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Decorin in Orbital Fibroblasts as a Possible Target in the Pathogenesis of Graves' Ophthalmopathy

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Abstract

Background: The initiation of Graves' ophthalmopathy (GO), an orbital manifestation of Graves' disease (GD), still remains unclear. Whether thyroid stimulating hormone receptor (TSHr) plays a pivotal role is still debatable. In context, fibroblast-derived proteins are currently under intense consideration.

Results: Current study was designed to evaluate whether decorin, a 38 kDa proteoglycan, could be a plausible target in the pathogenesis of GO utilising *in silico* methods for protein and sequence analysis. This fibroblast-derived protein is known to have conserved leucine rich repeats (LRR) and is constitutively expressed in fibroblasts and tissues of mesenchymal origin, unlike TSHr. Furthermore, decorin expression in orbital fibroblasts (OF), the target tissue implicated in GO, has not been investigated yet.

Conclusion: For the first time, this study reports a structural similarity of decorin to TSHr-α sub-unit. Thereby, indicating a possible involvement in GO pathogenesis. In conclusion, the collated data reported herein, is suggestive of decorin as a new candidate underlying GO pathogenesis.

Keywords: Graves' diseases; Graves' ophthalmopathy; Decorin; Orbital fibroblast; TSHr

Abbreviations:

GD: Graves' Disease; GO: Graves' Ophthalmopathy; OF: Orbital Fibroblasts; DF: Dermal Fibroblasts; TSHr: Thyroid Stimulating Hormone Receptor

Introduction

Graves' ophthalmopathy (GO) is a complex inflammatory, sight-threatening condition of the eye, which as the name suggests, is a thyroid-associated ophthalmopathy (TAO) often diagnosed in patients with Graves' hyperthyroidism or Graves' disease (GD) [1,2]. Although the precise pathophysiology of GO remains unclear, the loss of peripheral immune tolerance to the thyroid stimulating hormone receptor (TSHr) expressed in the thyroid follicular cells has been established as the underlying cause for hyperthyroidism in GD [2,3]. In the past decade, a unified hypothesis that assumes the circulating antibodies against TSHr (TSHr-Ab) to be the connecting link between GD and the development of its extra-thyroidal manifestation in the orbital tissue i.e., GO, is under intense study [2,4].

Though orbital fibroblasts (OF) are strongly implicated as the target site involved in the pathomechanism of GO, the expression profile of the target antigen TSHr remains elusive [5,6]. Studies have shown low abundance of TSHr mRNA in orbital connective tissues and cultured fibroblasts obtained from GO patients [7,8], which concurrently has been contradicted by other contemporary studies [9,10]. In addition, several studies demonstrated that the cultured OF from GO patients express detectable TSHr mRNA/protein, only after adipocyte differentiation was triggered *in vitro* or in severe conditions of GO [11-14]. Nevertheless, these studies unanimously agree that TSHr expression level is undetectable in orbital fibroblasts or orbital connective tissue samples from healthy donors [7,8,10,11,15].

Evidently, TSHr is not constitutively expressed in the said extra-thyroidal tissues, and therefore it becomes seemingly difficult to allocate it as a target antigen for the circulating TSHr-Abs in GD patients that might trigger the GO symptoms. However, TSHr expression in diseased orbital tissue or cultured fibroblasts post-adipocytic differentiation precedes the observation that high titres of TSHr-Ab positively correlate with severity of GO. In contrast, whether TSHr is involved in the 'initiation' of GO pathogenesis still remains inconclusive.

High resolution (2.55 Å) crystal structure of TSHr/TSHr-Ab complex was determined identifying amino acid 1-260 of the TSHr-α sub-unit (TSHr260) with a conserved LRR region, as the region recognized by the fragment antigen-binding (Fab) domain of TSHr-Ab [16,17]. These were also indicated in Graves' patients and animal models of the disease [2,4,5,18]. There are clinical conditions where GO occurs in absence of circulating TSHr antibodies, a condition termed 'euthyroid' [19]. T lymphocytes associated with OF, taken from 21 euthyroid GO patients were demonstrated to proliferate *in vitro* in presence of autologous orbital fibroblast proteins [20]. Subsequently, collagen XIII has been implicated as a target antigen and marker in orbital myopathies, especially TAO [19,21]. This coupled with the undetectable expression of TSHr in the target OF cells, indicates the

involvement of other putative fibroblast-expressed protein(s) in the pathomechanism of GO.

A small dermatan proteoglycan, decorin, expressed in corneal fibroblasts, belongs to the small leucine rich protein (SLRP) family [22]. The core protein (38 kDa) harbours a potent LRR region, which could be comparable to the TSHr-α sub-unit16.

Though decorin has been demonstrated to be secreted by dermal fibroblasts *in vitro*, there is no evidence or studies that show its expression in OF nor a structural comparison to TSHr [23]. Since, putative fibroblast-derived proteins are currently being considered to be involved in GO pathogenesis, this study for the first time, attempts to reveal the importance of decorin in this context. The structural homology of decorin with respect to TSHr-α sub-unit has been conducted and reported herein.

Methodology

In silico protein-structure analysis

A protein homology search via Basic Local Alignment Search Tool (BLAST) from NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) with the first 260 amino acids from TSHr (NCBI Reference sequence: P16473.2) was performed.

Further on, to analyse the relationship in more detail a protein-protein sequence alignment of decorin and TSHr260 with the help of ClustalW2 (a tool from EMBL-EBI - http://www.ebi.ac.uk/services which exhibits all identical amino acids) was conducted.

Since, functional relevance is more attributed to the secondary structure than the primary sequence; a structural comparison was performed further with the two protein structures. A 3-dimensional structure of both the proteins were derived from the protein data bank (http://www.rcsb.org) as a PDB file and analysed with the help of PyMOL from Schrodinger, LLC. for theoretical binding experiments Clus-pro *in silico* docking simulations have been used to show that the decorin core protein and TSHr-α sub-unit could have similar docking site to the TSHr-Ab.

Results

In silico

A P-BLAST search with TSHr260 sequence (260 amino acids) against the reference sequence protein database revealed that decorin (NCBI accession number NP_001911.1), a protein expressed by fibroblasts, has 25% identical amino acids with the former, most of them occurring within the C-terminal end of TSHr260 (residues 200-246) (Figure 1). VAST search, a secondary structure comparison, tool among the known 3D structure database also revealed TSHr260 sharing structural homology with decorin (PDB ID 1XEC, chain A) (Figure 2), which was then selected as a suitable candidate.

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Decorin MKATIILLLLAQVSWAGPFQQRGLFDFMLEDEASGIGPEVPDDRDFEPSLGPVCPFRCQC 60
TSHR260 --------------------------------DVSQTSVTALPSKGLEHLKELIARNTWTL 29
 * *
Decorin HLRVVQCSDLGLDKVPKDLPPDTTLLDLQNNKITEIKDGDFKNLKNLHALILVNNKISKV 120
TSHR260 KKLPLSLSFLHLTRADLSYPSHCCAFKNQK-KIRGILESLMCNESSMQSLRQRKSVNALN 88
 * * * * * ** * * *
Decorin SPGAFTPLVKLERLYLSKNQLKELPEKMPKTLQELRAHENEITKVRKVTFNGLNQMIVIE 180
TSHR260 SP---------------------LHQEYEENLGDSIVGYKEKSKFQDTHNN-AHYYVFFE 126
 ** * * * * * *
Decorin LGTNPLKSSGIENGAFQGMKKLSYIRIADTNITSIPQGLPPSLTELHLDGNKISRVDAAS 240
TSHR260 EQEDEIIGFGQELKNPQEETLQAFDSHYDYTICGDSE-------------DMVCTPKSDE 173
 * * * * *
Decorin LKGLNNLAKLGLSFNSISAVDNGSLANTPHLRELHLDNNKLTRVPGGLAEHKYIQVVYLH 300
TSHR260 FNPCEDIMGYKFLRIVVWFVSLLALLGNVFVLLILLTSHYKLNVPR----------FLM 222
 * * * ** *
Decorin NNNISVVGSSDFCPPGHNTKKASYSGVSLFSNPVQYWEIQPSTFRCVYVRSAIQLGNYK 359
TSHR260 CN----LAFADFCMG---MYLLLIASVDLYT-------------HSEYYNHAIDWQTG- 260
 * *** * * * **
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Figure 1: Primary homology of TSHr260 and decorin. Protein-protein sequence alignment of decorin (NCBI Reference Sequence: NP_001911.1) and TSHr260 created from full lengths TSHr (NCBI Reference Sequence: P16473.2). ClustalW2 was used as alignment tool, * shows identical amino acids.

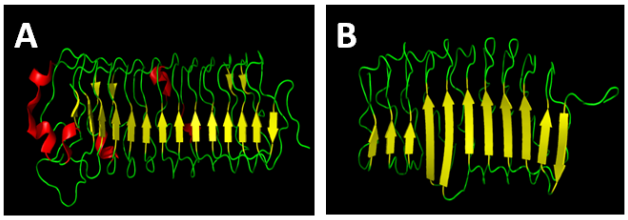

Figure 2: Secondary structural homology of TSHR260 and decorin. A) Bovine decorin crystal structure with beta-sheets (yellow) and alpha-helices (red) modified from DOI:10.2210/pdb1xec/pdb B) TSHR260 crystal structure containing amino acid sequence 22-260 with beta-sheets (yellow) modified from DOI:10.2210/pdb1xec/pdb. Data for both crystal structures were obtained from protein data bank and analysed and modified with PyMOL from Schrodinger, LLC.

The biological effects of decorin are reported to be mediated via its core protein, with the binding sites for TGF-β and IGF-1R mapped to distinct LRR motifs in the interior region located within the concave face of decorin. Clus-pro *in silico* (Figure 3) data suggests that the concave LRR region could be a potential site of identification for circulating TSHr-Ab that bind to the LRR site of TSHr-α. The structural similarity and conserved LRR regions between decorin and TSHr-α via PyMOL (Figure 4), presented a possibility of an interaction of TSHr-Ab with decorin instead of the original, TSHr.

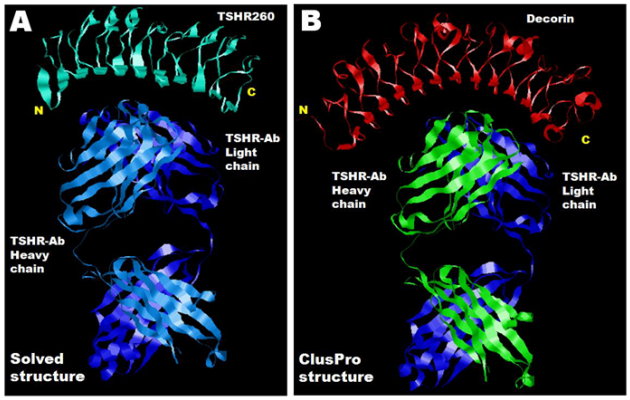

Figure 3: Structural homology of TSHr-α sub-unit and decorin. A) Crystal structure of TSHR-Ab (blue) with TSHR-α sub-unit depicting 1-260 amino acid sequence (cyan) containing the LRR repeats, as reported by Sanders et al. [16]. B) ClusPro-generated structure of decorin core protein (red) containing the LRR region.

Discussion

Pathogenesis of GO and its association with thyroiditis still remains elusive. Though TSHr is heavily implicated and studied in regards to GO2, 5, search for putative antigens and target proteins in orbital fibroblasts is currently apparent19. For the first time, this study reports the structural similarity of decorin with TSHr-α sub-unit.

In silico observation of a protein sequence alignment similarity and LRR conserved regions (Figures 4a and 4b) between decorin and TSHr-α sub-unit insinuates a novel target candidate for GO pathogenesis and is discussed hereafter.

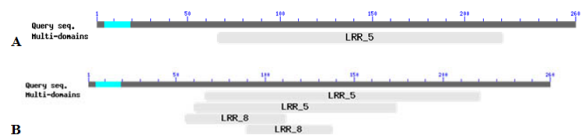

Figure 4: Modified conserved LRR regions between decorin and TSHr-α A) TSHR260 [32] and B) decorin from homo sapiens with a length of 347 amino acids [33].

One of the early studies investigating the expression of TSHr in retro-orbital connective tissue and cultured fibroblasts revealed low abundance of a TSHr variant mRNA, corresponding to the extra-cellular domain, in samples derived from GO patients, unlike those of healthy donors [7]. The same group demonstrated low TSHr expression in GO orbital tissues and cultured fibroblasts with alternative protocols like liquid hybridisation and southern blots [8]. On the contrary, in another study, real-time PCR failed to show statistically significant difference for TSHr expression between GO and healthy orbital tissues [10], which further agreed with the PCR studies of Paschke et al. [9]. Though conflicting, TSHr expression has been reported in the first few passages of GO OF cells previously [8].

Nevertheless, it conforms to the observations of Valyasevi et al. where GO OF grown in Medium 199 failed to show express TSHr even though the tissue explant did [11].

Culp et al. [23] and Pulkkinen et al. [24] comfirms the presence of decorin in cultured dermal fibroblasts and absence in Hela cell.

Subsequently, significant increase in both mRNA (qRT-PCR) and protein content with increasing time in culture was demonstrated for GO derived OF cells, in contrast to non-GO OF. Though it can be argued that earlier passages of primary donor cells are more representative of the native phenotype, it is interesting to see that only the diseased OF cells possess the cellular machinery that up-regulates the decorin expression significantly, in an isolated *in vitro* system without influence of humoral factors. An earlier study by Bahn and co-workers, showed days in culture affected the TSHr mRNA content in cultured orbital fibroblasts, which could be detected only in the first four passages [8]. The TSHr expression was attributed to the presence of lipid-laden adipocytes. In fact studies have confirmed that the expression of TSHr occurs on adipocytic differentiation which are usually apparent in the late stages of the disease, when the initial autoimmune attack has disappeared [3,10-13]. It would be interesting to note whether the variation in decorin expression with increasing passage numbers could be attributed to the gradual loss of adipocytes from the population of GO OF cells *in vitro*. This could further evaluate whether increasing levels of decorin in the undifferentiated OF could be an early biomarker for the disease. Nevertheless, further investigations in undifferentiated GO OF cells and normal OF cells is required to assess any plausible role of decorin in the initiation of GO symptoms.

Decorin is a concave-shaped SLRP (359 amino acids) present in the extra cellular matrix and pericellular region of all connective tissues [22]. It contains a core protein with 10 to 12 LRRs covalently bound to a chondroitin sulphate GAG side chain on its N-terminus [22]. Importantly, decorin have been shown to bind with TGF-β and antagonize its effect on myofibroblast differentiation and expression of profibrotic genes by ocular fibroblasts [25,26]. In addition to its suppressive effect on myofibroblast formation, decorin is known to interact with IGF-1R with high affinity comparable to the natural ligand IGF-1, but instead causes IGF-1R down-regulation and subsequent degradation [27,28]. Furthermore, decorin also binds to and sequesters the IGF-1 with lower affinity indicating that decorin could regulate adipogenesis as well [27]. The physiological relevance of decorin is further reinforced in experimental animal models of corneal inflammation and renal fibrosis, where decorin deficiency resulted in a significant increase in IGF-1R levels and TGF-β induced effects phenotypes similar to those exhibited by GO OF *in vivo* [2,5,15,19,25,29]. Increased expression of decorin in GO OF cells could lead to its secretion and sequestration of in the ECM and pericellular space. This invariably could lead to or worsen oedema as the negatively charged GAG side chains of decorin are potent water binders [30,31]. These evidence taken together suggest that decorin may be capable of directly regulating pathogenic outcomes relevant in GO such as myofibroblast differentiation, fibrosis, adipogenesis and oedema.

Unlike the restricted expression pattern of TSHr, decorin can be found in multiple tissues [22,24] including dermal fibroblasts [23].

Conclusion

In conclusion, the current study demonstrated for the first time that decorin has structural similarity to TSHr-α sub-unit. It is also

constitutively expressed in orbital fibroblasts. An attempt was made to evaluate its involvement in GO pathogenesis via *in silico* tools compared to the known expression profile between diseased vs. healthy cells. Clus-pro *in silico* docking hinted also that the core protein of decorin could have similar docking site to the TSHr-Ab. Figure 3 summarises and compares the study by Sanders and co-workers and our observations which hinted at the importance of decorin as a candidate fibroblast 'antigen' [16,32,33].

For studies in the field of GO is the difficult accessibility to orbital tissues from patients as they are obtained mostly at the time of surgical interventions. Therefore, the presented data requires thorough investigation in clinical samples to confirm the significance of decorin in GO pathogenesis. Nevertheless, a novel approach was undertaken to understand the role of decorin in respect to GO, which subsequently could pave way for improved therapeutic interventions.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

LH, KM and CO jointly designed and carried out the in silico protein-structure analysis.

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