

# Eye-opening potential for tetraspanin Tspan12 as a therapeutic target for diseases of the retinal vasculature

Tomlinson, Michael

DOI:

[10.1161/CIRCULATIONAHA.117.028521](https://doi.org/10.1161/CIRCULATIONAHA.117.028521)

License:

None: All rights reserved

*Document Version*

Peer reviewed version

*Citation for published version (Harvard):*

Tomlinson, M 2017, 'Eye-opening potential for tetraspanin Tspan12 as a therapeutic target for diseases of the retinal vasculature', *Circulation*, pp. 196-199. <https://doi.org/10.1161/CIRCULATIONAHA.117.028521>

[Link to publication on Research at Birmingham portal](#)

**Publisher Rights Statement:**

Final Version of Record available at: <http://dx.doi.org/10.1161/CIRCULATIONAHA.117.028521>

Publication details verified 9/8/17

**General rights**

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

**Take down policy**

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.

*Circulation*

**EDITORIAL**

**Eye-opening potential for tetraspanin Tspan12 as a therapeutic target for  
diseases of the retinal vasculature**

**Michael G. Tomlinson, DPhil**

**Affiliation:** School of Biosciences  
College of Life and Environmental Sciences  
University of Birmingham  
Birmingham B15 2TT, UK

**Running title:** Anti-Tspan12 therapy for retinal vascular disease

**Correspondence to:** Dr Michael G. Tomlinson  
School of Biosciences  
College of Life and Environmental Sciences  
University of Birmingham  
Birmingham B15 2TT, UK  
E-mail [m.g.tomlinson@bham.ac.uk](mailto:m.g.tomlinson@bham.ac.uk)  
Telephone +44 121 414 2507  
FAX +44 121 414 5925

Age-related macular degeneration is the leading cause of blindness in industrialized nations, affecting over 10 million Americans. The most severe form of macular degeneration, the ‘wet’ form, is caused by growth of abnormal vessels which leak excessive fluid under the retina, damaging the macula and leading to loss of sharp, central, color vision. Vascular endothelial growth factor (VEGF) is a major factor that drives vasoproliferative diseases such as macular degeneration, and antibodies to VEGF are a mainstay of treatment. In this issue of *Circulation*,<sup>1</sup> Bucher and colleagues identify tetraspanin Tspan12 as a therapeutic target for retinal vascular proliferation, and they invent a Tspan12-targeting therapy.

The retinal vasculature is uniquely adapted to supply oxygen and nutrients to photoreceptor cells without blocking in-coming light. However, these specialised features also make the retinal vasculature susceptible to vasoproliferative retinopathy, which is the aberrant growth of new blood vessels and breakdown of their barrier function.<sup>2</sup> Vasoproliferative retinopathies are driven by VEGF, a key regulator of physiological and pathological angiogenesis. VEGF production is driven primarily by the hypoxia-inducible factor (HIF) family of transcription factors. VEGF stimulates angiogenesis by interacting with its receptors VEGFR1 and VEGFR2, which in turn activate intracellular signaling cascades such as the RAS-RAF-MAPK pathway, triggering endothelial migration and proliferation. VEGF-induced VEGFR2 signaling also increases vascular permeability by separating VE-cadherin complexes and intercellular adhesion junctions. The most effective treatment for vasoproliferative retinopathies is anti-VEGF therapy delivered by periodic intravitreal injections. However, the lack of response in some patients, and relapses of neovascularisation and edema between treatments, indicates the clinical need for novel treatments.<sup>2</sup>

In the current issue of *Circulation*,<sup>1</sup> Bucher and colleagues discovered that tetraspanin Tspan12 is a new therapeutic target for vasoproliferative retinopathies. The tetraspanins are a superfamily of 33 integral membrane with four transmembrane regions. Tetraspanins interact with specific partner proteins and regulate their intracellular trafficking and/or lateral diffusion and clustering at the cell surface.<sup>3,4</sup> On endothelial cells, for example, tetraspanin CD151 is important for angiogenesis, potentially by promoting signaling through its laminin-binding integrin partner proteins. In addition, tetraspanins CD9, CD63 and CD151 promote leucocyte capture during inflammation by clustering and forming ‘sticky platforms’ of the adhesion molecules intercellular adhesion molecule-1 (ICAM-1), P-selectin and vascular cell adhesion molecule-1 (VCAM-1), respectively.<sup>5</sup> However, a substantial proportion of the 33 human tetraspanins have not been functionally characterized in terms of regulation of specific partner proteins at the molecular level. This is in part due to a lack of effective antibodies to these tetraspanins.

In recent advances to the tetraspanin field, super-resolution imaging has shown that tetraspanins exist as nanodomains on the cell surface that comprise clusters of approximately 10 tetraspanins of a single type.<sup>6</sup> Moreover, the first full-length crystal structure of a tetraspanin, CD81, reported last year,<sup>7</sup> revealed a cone-shaped appearance with a cholesterol-binding cavity within the transmembrane regions (Figure 1). Molecular dynamics simulations suggest that removal of the cholesterol may induce a striking conformational change in which the large extracellular region swings upwards, providing a potential mechanism by which tetraspanins could regulate partner proteins by acting as molecular switches.<sup>7</sup> While this is unproven and based on just one tetraspanin structure, it nevertheless raises the exciting possibility that tetraspanins could be therapeutically targeted by small molecules that affect the cholesterol-binding cavity, or antibodies/small molecules that lock tetraspanins into a particular conformation.

In 2009, Junge and colleagues reported the first characterization of Tspan12-deficient mice and showed Tspan12 to be important for development of the retinal vasculature by promoting  $\beta$ -catenin signaling,<sup>8</sup> an important pathway in embryonic development and in adult health and disease. At the molecular level, Tspan12 appears to promote multimerization of the Norrin receptor Frizzled-4 (FZD-4)<sup>8</sup> and interaction between FZD4 and its co-receptor low-density lipoprotein receptor-related protein 5 (LRP5) (Figure 1).<sup>9</sup> In response to Norrin, the resulting intracellular signal stabilizes the multi-functional cytoplasmic protein  $\beta$ -catenin, which can then translocate to the nucleus to act as a transcriptional co-activator and turn on the expression of target genes<sup>10</sup> (Figure 1). Based on their observations in mice, Junge and colleagues speculated that mutations in human Tspan12, like existing mutations in FZD4, LRP5 and Norrin, might lead to the blinding disease familial exudative vitreoretinopathy.<sup>8</sup> This was proved correct a year later, with the identification of disease-causing Tspan12 mutations.<sup>11, 12</sup> It is worth noting that Tspan12 expression in the retina is restricted to endothelial cells,<sup>8</sup> but in the rest of the body expression is more widespread and Tspan12 on epithelial cells and fibroblasts has been implicated in cancer progression via FZD4/LRP5-mediated  $\beta$ -catenin signaling.<sup>9, 13</sup>

In the present study, Bucher and colleagues aimed to generate anti-Tspan12 antibodies and test whether these could reduce FZD4/LRP5-mediated  $\beta$ -catenin signaling and so be used as a treatment for vasoproliferative retinopathy.<sup>1</sup> Historically, attempts to generate new tetraspanin antibodies have often proved unsuccessful, possibly due to the small size of tetraspanins and high degree of protein sequence identity between human and mouse (98% for Tspan12). The Bucher approach to this problem was novel and innovative.<sup>1</sup> A 48 amino acid peptide encompassing the variable region of Tspan12 was generated and used as a target antigen. This variable region forms part of the large extracellular region, contains all six of the structurally-important cysteine residues, is relatively divergent in sequence between

tetraspanins and typically mediates interactions with partner proteins.<sup>3</sup> A phage library of approximately  $10^9$  human antibodies was then screened against the peptide. This yielded six high-affinity antibodies. One of these was definitively shown to recognize full-length Tspan12 by flow cytometry and immunofluorescence microscopy, using Tspan12-over-expressing and Tspan12-knockdown cells as key controls.<sup>1</sup> In future, it will be interesting to determine whether this approach will be applicable to other tetraspanins.

The chosen Tspan12 antibody was found to have only a modest inhibitory effect on primary human umbilical vein endothelial cell function in vitro, using assays for migration and cell-cell adhesion.<sup>1</sup> However,  $\beta$ -catenin expression was substantially reduced by antibody treatment, suggesting inhibition of  $\beta$ -catenin signaling. The mechanism appears to involve disruption of Tspan12 interaction with FZD4, as measured by a modest reduction in co-immunoprecipitation of the proteins from transfected cells.<sup>1</sup> While this is an artificial system, it may actually under-represent the true disrupting effect of the Tspan12 antibody, since much of the Tspan12-FZD4 complex may be intracellular and so unaffected by antibody treatment. Tetraspanin antibody-mediated disruption of tetraspanin/partner interactions is not without precedent, since a CD151 antibody can impair laminin-binding integrin function by disrupting the CD151-integrin interaction.<sup>14</sup> Nevertheless, to support the proposed mechanism of Tspan12 antibody action, future studies would benefit from fluorescence-based imaging methods that can measure protein-protein interactions in living cells in real time.

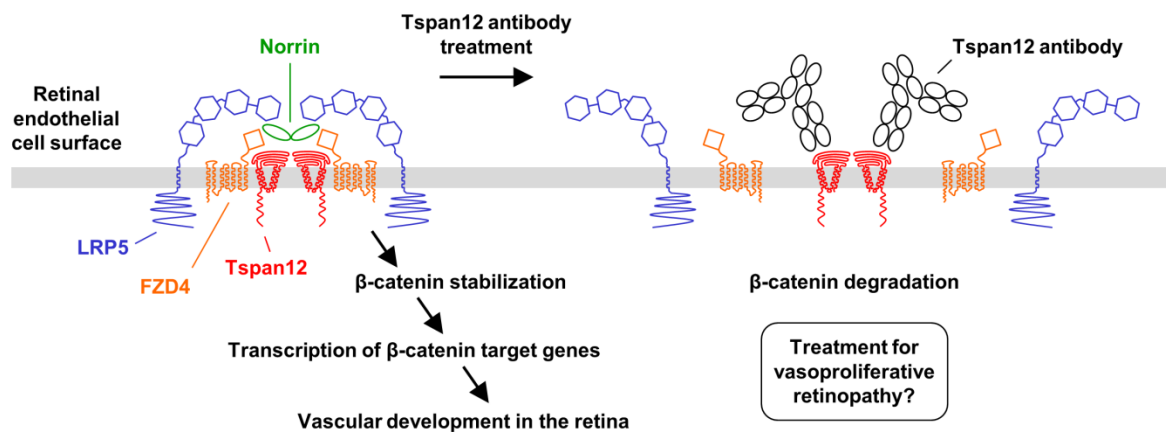
To investigate the therapeutic potential of their Tspan12 antibody, Bucher and colleagues used two mouse vascular retinopathy models, the oxygen-induced retinopathy model and the very low density lipoprotein receptor (VLDLR) knockout model.<sup>1</sup> Oxygen-induced retinopathy is the most widely used model for disease of the inner retinal vasculature, through which light must pass to reach the photoreceptor cells. In this model, mice are

subjected to 75% oxygen from 7 to 12 days of age, which suppresses VEGF expression in the retina with consequent loss of blood vessels. Upon re-exposure to normal air, the retina suffers relative hypoxia due to the lack of blood vessels. The resulting high-level expression of VEGF causes vessel overgrowth and characteristic vascular tufts which are maximal on day 17 of age, before this repairs during the following several days. In this model, intravitreal injection of Tspan12 antibody at day 12 of age reduced aberrant vessel overgrowth at day 17 by approximately one third, whilst promoting recovery of the lost blood vessels.<sup>1</sup> Thus the antibody modulates vessel growth rather than functioning as an anti-angiogenic. The second model was the VLDLR knockout mouse, in which neovascular tufts develop from the outer retinal vessels that supply the over-lying photoreceptor cells. Similar to its efficacy in the previous model, intravitreal injection of the Tspan12 antibody reduced neovascular tuft formation by approximately one third.<sup>1</sup>

The gold standard treatments for vascular retinopathies are anti-VEGF agents such as Aflibercept, a clinically-approved recombinant protein derived from VEGF receptors 1 and 2 that neutralizes VEGF via high-affinity binding. In their concluding experiments, Bucher and colleagues used the oxygen-induced retinopathy model to show that co-treatment with Aflibercept and Tspan12 antibody doubled the efficacy of either treatment alone.<sup>1</sup> Titration experiments further showed that relatively low dose Aflibercept, in combination with Tspan12 antibody, is an effective treatment without inducing negative side-effects on retinal function that are evident with higher doses of Aflibercept.<sup>1</sup>

In summary, this exciting study will be an ‘eye-opener’ for ophthalmologists, prompting clinical trials to assess anti-VEGF and Tspan12 antibody combination therapy for vascular retinopathies such as age-related macular degeneration and diabetic retinopathy. This study may also galvanize research into the molecular mechanism by which Tspan12

regulates FZD4, and in particular whether the recently-proposed conformational change in tetraspanins could impact on FZD4/LRP5-induced  $\beta$ -catenin signaling.



**Figure 1. Hypothetical model to show the role of Tspan12 in Norrin-induced FZD4/LRP5/ $\beta$ -catenin signaling and inhibition by the Tspan12 antibody.**

## SOURCES OF FUNDING

Dr Tomlinson's research on tetraspanins in the vasculature is funded by a British Heart Foundation Project Grant (PG/13/92/30587) and PhD Studentship (FS/12/79/29871).

## DISCLOSURES

None.



## REFERENCES

1. Bucher F, Zhang D, Aguilar E, Sakimoto S, Diaz-Aguilar S, Rosenfeld M, Zha Z, Zhang H, Friedlander M and Yea K. Antibody-Mediated Inhibition of Tspan12 Ameliorates Vasoproliferative Retinopathy Through Suppression of beta-Catenin Signaling. *Circulation*. 2017.
2. Campochiaro PA. Molecular pathogenesis of retinal and choroidal vascular diseases. *Prog Retin Eye Res*. 2015;49:67-81.
3. Charrin S, Jouannet S, Boucheix C and Rubinstein E. Tetraspanins at a glance. *J Cell Sci*. 2014;127:3641-3648.
4. Hemler ME. Tetraspanin proteins promote multiple cancer stages. *Nat Rev Cancer*. 2014;14:49-60.
5. Bailey RL, Herbert JM, Khan K, Heath VL, Bicknell R and Tomlinson MG. The emerging role of tetraspanin microdomains on endothelial cells. *Biochem Soc Trans*. 2011;39:1667-1673.
6. Zuidsherwoude M, Gottfert F, Dunlock VM, Figdor CG, van den Bogaart G and van Sriel AB. The tetraspanin web revisited by super-resolution microscopy. *Sci Rep*. 2015;5:12201.
7. Zimmerman B, Kelly B, McMillan BJ, Seegar TC, Dror RO, Kruse AC and Blacklow SC. Crystal Structure of a Full-Length Human Tetraspanin Reveals a Cholesterol-Binding Pocket. *Cell*. 2016;167:1041-1051.
8. Junge HJ, Yang S, Burton JB, Paes K, Shu X, French DM, Costa M, Rice DS and Ye W. TSPAN12 regulates retinal vascular development by promoting Norrin- but not Wnt-induced FZD4/beta-catenin signaling. *Cell*. 2009;139:299-311.

9. Knoblich K, Wang HX, Sharma C, Fletcher AL, Turley SJ and Hemler ME. Tetraspanin TSPAN12 regulates tumor growth and metastasis and inhibits beta-catenin degradation. *Cell Mol Life Sci.* 2014;71:1305-1314.
10. Driehuis E and Clevers H. WNT signalling events near the cell membrane and their pharmacological targeting for the treatment of cancer. *Br J Pharmacol.* 2017; doi: 10.1111/bph.13758.
11. Nikopoulos K, Gilissen C, Hoischen A, van Nouhuys CE, Boonstra FN, Blokland EA, Arts P, Wieskamp N, Strom TM, Ayuso C, Tilanus MA, Bouwhuis S, Mukhopadhyay A, Scheffer H, Hoefsloot LH, Veltman JA, Cremers FP and Collin RW. Next-generation sequencing of a 40 Mb linkage interval reveals TSPAN12 mutations in patients with familial exudative vitreoretinopathy. *Am J Hum Genet.* 2010;86:240-247.
12. Poulter JA, Ali M, Gilmour DF, Rice A, Kondo H, Hayashi K, Mackey DA, Kearns LS, Ruddle JB, Craig JE, Pierce EA, Downey LM, Mohamed MD, Markham AF, Inglehearn CF and Toomes C. Mutations in TSPAN12 cause autosomal-dominant familial exudative vitreoretinopathy. *Am J Hum Genet.* 2010;86:248-253.
13. Otomo R, Otsubo C, Matsushima-Hibiya Y, Miyazaki M, Tashiro F, Ichikawa H, Kohno T, Ochiya T, Yokota J, Nakagama H, Taya Y and Enari M. TSPAN12 is a critical factor for cancer-fibroblast cell contact-mediated cancer invasion. *Proc Natl Acad Sci USA.* 2014;111:18691-18696.
14. Nishiuchi R, Sanzen N, Nada S, Sumida Y, Wada Y, Okada M, Takagi J, Hasegawa H and Sekiguchi K. Potentiation of the ligand-binding activity of integrin alpha3beta1 via association with tetraspanin CD151. *Proc Natl Acad Sci USA.* 2005;102:1939-1944.