Received: 12 October 2023 | Revised: 18 October 2023 | Accepted: 19 October 2023

https://doi.org/10.1016/j.rpth.2023.102249

rpth research & practice in thrombosis & haemostasis

RESEARCH LETTER

Western blot and immunohistochemical analysis of mouse tissue factor

Rigor and reproducibility are the cornerstones of science. An expectation is that the methods in a published article provide accurate information to allow data to be reproduced by other laboratories. There are many journals publishing scientific papers that rely on reviewers and editors to ensure the quality of the published paper. Unfortunately, there is no system to maintain scientific standards in these journals.

The International Working Group for Antibody Validation highlighted the need for validating antibodies used in research [1]. Recommendations included performing comparative studies with antibodies that bind to the target protein at nonoverlapping epitopes and eliminating or reducing expression of the target protein through genome editing or RNA interference. Commercial antibodies should be independently validated.

Zhao et al. [2] investigated the role of tissue factor (TF) in epithelial-mesenchymal transition of bronchial epithelial cells in a mouse model of allergic asthma using house dust mites. The authors evaluated the ability of short hairpin RNAs (shRNAs) to knock down TF expression in human cells and mice. TF mRNA expression was measured using reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Mouse TF protein expression was assessed by western blotting using a rabbit anti-human TF antibody from Abcam (Ab104513). Although not stated in the paper, we presume that the same antibody was used for immunohistochemistry. Abcam recommends this antibody for detection of human TF by western blotting and immunohistochemistry. It is predicted by Abcam to cross-react with rat and mouse TF. One of the shRNAs (shRNA2) was then selected to determine the effect of TF knockdown in mouse lungs, and the level of reduction of mouse TF expression was assessed by RT-qPCR, western blotting, and immunohistochemistry.

We identified several major issues with the manuscript. We wrote a letter to the Editor of *International Immunopharmacology* about our concerns with the antibody used by the authors. The Editor dismissed our concern and told us that the authors also measured levels of TF mRNA. Therefore, we checked the sequence of the primers used for RT-qPCR and the shRNAs. Since the authors used both human cells and mice, there should have been 2 sets of RT-qPCR primers and 2 sets of shRNAs. However, there was only 1 set of RT-qPCR primers and 1 set of shRNAs in the paper. We found that the shRNAs matched the sequence of human transferrin and not human or mouse TF. The RT-qPCR primers matched mouse TF mRNA [2]. Transferrin and TF often get confused. The gene name for transferrin is *TF*, whereas the gene name for TF is *F3*. A recent study analyzed the role of the TF-protease-activated receptor-1 pathway in a mouse model of liver injury and mistakenly used an antibody against transferrin (ProteinTech, 17435-1-AP) for western blotting and immunohistochemistry instead of an anti-TF antibody [3]. We submitted a commentary on this to Frontiers in Pharmacology, but the journal selected to withdraw the commentary because the journal deemed that a "commentary should further scientific discourse and contribute to scholarly debate on a topic." (Mackman N and Luyendyk J). Zhao et al. published a corrigendum with new shRNAs that knockdown mouse TF mRNA expression and new RT-qPCR primers to measure human TF mRNA expression [4]. However, no information was provided on the shRNAs used to knock down human TF mRNA. In addition, the corrigendum did not address our concerns with the antibody used in the study.

We assessed the ability of 4 different commercial antibodies to detect mouse TF in cell lines by western blotting [5]. Importantly, we found that the Abcam antibody Ab104513 used by Zhao et al. did not detect TF in cell lysates by western blotting. In contrast, a goat anti-mouse TF antibody from R&D Systems (AF3178) detected TF in the cell lysate by western blotting [4]. We also used AF3178 to demonstrate that TF protein expression is increased in the bronchoalveolar fluid collected from the lungs of mice sensitized and challenged with ovalbumin [6].

Abcam states that Ab104513 can be used for immunohistochemistry. However, in the light of the western blot data with this antibody, use of Ab104513 for immunohistochemistry should be independently validated with appropriate controls. We used AF3178 to detect TF expression by immunohistochemistry in the lungs of mice with or without influenza A infection [7]. TF expression was increased in epithelial cells in the lungs of mice infected with influenza A. Importantly, we observed reduced TF expression in epithelial cells in $TF^{fl/fl}$, SPC^{cre} mice, which have a selective deletion of TF in lung epithelial cells.

© 2023 The Author(s). Published by Elsevier Inc. on behalf of International Society on Thrombosis and Haemostasis. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

In conclusion, despite the corrigendum, Zhao et al. [4] still have not provided information on the shRNAs used to knock down human TF mRNA expression. In addition, our concerns about the use of antibody Ab104513 were not addressed. We recommend using antibody AF3178 to measure mouse TF by western blotting and immunohistochemistry.

FUNDING

This work was not supported by any funding.

AUTHOR CONTRIBUTIONS

N.M. wrote the manuscript. The coauthors provided helpful comments and revised the manuscript.

RELATIONSHIP DISCLOSURE

The authors have no relevant conflicts of interest to disclose.

Nigel Mackman[1]
Yohei Hisada[1]
Jin-Ah Park[2]

[1]*Department of Medicine, Division of Hematology, UNC Blood Research Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA*
[2]*Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA*

Handling Editor: Prof Yotis Senis

Correspondence

Nigel Mackman, Department of Medicine, University of North Carolina at Chapel Hill, 116 Manning Drive, Mary Ellen Jones Building, Room 8004B, Campus Box 7035, Chapel Hill, NC 27599, USA.
Email: nmackman@med.unc.edu

REFERENCES

[1] Uhlen M, Bandrowski A, Carr S, Edwards A, Ellenberg J, Lundberg E, et al. A proposal for validation of antibodies. *Nat Methods*. 2016;13:823–7.

[2] Zhao J, Jiang T, Li P, Dai L, Shi G, Jing X, et al. Tissue factor promotes airway pathological features through epithelial-mesenchymal transition of bronchial epithelial cells in mice with house dust mite-induced asthma. *Int Immunopharmacol*. 2021;97:107690. https://doi.org/10.1016/j.intimp.2021.107690

[3] Jing-Lun Z, Shuang C, Li-Mei Z, Xiao-Dong L. YKL-40 promotes chemokine expression following drug-induced liver injury via TF-PAR1 pathway in mice. *Front Pharmacol*. 2023;14:1205062. https://doi.org/10.3389/fphar.2023.1205062

[4] Zhao J, Jiang T, Li P, Dai L, Shi G, Jing X, et al. Corrigendum to "Tissue factor promotes airway pathological features through epithelialmesenchymal transition of bronchial epithelial cells in mice with house dust mite-induced asthma". [Int. Immunopharmacol. 97 (2021) 107690]. Int Immunopharmacol 2022;108:108886. https://doi.org/10.1016/j.intimp.2022.108886

[5] Rosell A, Moser B, Hisada Y, Chinthapatla R, Lian G, Yang Y, et al. Evaluation of different commercial antibodies for their ability to detect human and mouse tissue factor by western blotting. *Res Pract Thromb Haemost*. 2020;4:1013–23.

[6] Mitchel JA, Antoniak S, Lee JH, Kim SH, McGill M, Kasahara DI, et al. IL-13 augments compressive stress-induced tissue factor expression in human airway epithelial cells. *Am J Respir Cell Mol Biol*. 2016;54:524–31.

[7] Antoniak S, Tatsumi K, Hisada Y, Milner JJ, Neidich SD, Shaver CM, et al. Tissue factor deficiency increases alveolar hemorrhage and death in influenza A virus-infected mice. *J Thromb Haemost*. 2016;14:1238–48.