# Transglutaminase-2: Nature's Glue in Lung Fibrosis?

Tissue fibrosis represents a failed wound repair event characterized by aberrant epithelial differentiation, persistent myofibroblast activation, and excessive extracellular matrix (ECM) deposition (1, 2). The fibrotic ECM has been recognized to be one of the primary drivers for disease progression as it not only compromises the normal tissue architecture, but also causes phenotypic dysregulation of many types of surrounding cells (2, 3). The buildup of the pathological ECM in the fibrotic tissue is believed to be caused by decreased ECM degradation as well as increased ECM production (2, 3). Studies in the past have discovered several families of enzymes, such as lysyl oxidases and transglutaminases, which mediate the crosslinking among various ECM proteins, especially collagen, fibronectin, and elastin in fibrotic tissues, thereby markedly enhancing the resistance of the fibrotic ECM to the degradation by various proteases (4, 5).

There are currently nine mammalian transglutaminase family members that have been discovered (6). Members of the transglutaminase family catalyze a calcium ($Ca^{2+}$)-dependent formation of ε-(γ-glutamyl) lysine bonds between a polypeptide or protein-bound glutamine and the ε-amino group of a polypeptide or protein-bound lysine, or between a polypeptide or protein-bound glutamine and a primary amine (e.g., spermidine). TG2 (transglutaminase 2), also known as tissue transglutaminase, is perhaps the most abundant and the best studied transamidating enzyme (7, 8). It is found in many cell types and is localized in multiple cellular compartments, including the nucleus, cytosol, mitochondria, endolysosomes, plasma membrane, and ECM (7, 8). TG2 has been implicated in tissue fibrosis, including in the lung, for many years. The supporting evidence includes the increased expression and activity of TG2 in fibrotic tissues, the protection from this pathology by TG2 ablation in mice, and more importantly, the potent therapeutic efficacy of TG2 inhibitors in treating this disease in animal models (8–10).

Although nonenzymatic functions have been proposed to contribute to the profibrotic effect of TG2 on the basis of its noncovalent interactions with a variety of cellular proteins, the primary focus is still on its enzymatic protein crosslinking activity (8). The fibrotic ECM is the most likely candidate among suspected TG2 substrates because crosslinks between the ECM components (collagens, fibronectins, and elastins) buttress their defense against protease degradation and thereby promote disease progression by preventing fibrotic resolution. However, this notion is largely supported by indirect and/or *in vitro* biochemical evidence (11). Given both the intra- and extracellular localization of TG2 in many types of lung cells (7, 8), it seems logical that additional intra- and extracellular proteins may also be subjected to TG2 modification. Identification of potential native TG2 substrates in lung fibrosis will likely improve our understanding of how TG2 promotes the pathogenesis.

In this issue of the *Journal*, Takeuchi and colleagues (pp. 319–330) used a clever approach to identify a number of TG2 substrates in pulmonary fibrosis (12). In the study, they first confirmed previous findings that TGKO (TG2 knockout) mice develop less lung fibrosis than wild-type (WT) animals after bleomycin injury (13). They show that WT mice demonstrated increased TG2 expression with elevated TG2 activity, as indicated by the increased incorporation of substrate peptide pepT26 in the fibrotic lungs. PepT26 is a preferred TG2 glutamine donor oligopeptide that was developed by the investigators in their prior studies (14). The pepT26 specificity for TG2 was further validated in the current study, as TG2 knockout mice showed a complete loss of the pepT26 incorporation in the injured lung. They also found that the increased transglutaminase activity in fibrotic lungs could be largely ascribed to TG2 because the cadaverine incorporation, which reflects the pan-transglutaminase activity, was markedly decreased in TG2 knockout mice.

Having shown the dominant status of TG2 over other transglutaminase members in the lung and the high specificity of pepT26 for TG2, they then incubated biotinylated pentylamine and pepT26 with freshly cut lung sections from mice treated with bleomycin and control mice, from which they purified proteins bound by the probes. This approach has the unique advantage over the alternative approach that uses whole tissue extracts because it is able to identify native substrates and minimizes false positive or negative incorporations because of the destruction of the normal cell and tissue structures. A mass spectrometry analysis was then used to identify the purified proteins, which was followed by post-proteomics data analysis.

Using the protein–protein interaction network analysis and a novel graphical theoretic clustering algorithm (MCODE), Takeuchi and colleagues identified six highly connected and distinct clusters in the protein–protein interaction network. The clusters confirmed some previous findings, such as the involvement of TG2 in the modification of collagens and integrins. Some of the enriched pathways have previously been reported in idiopathic pulmonary fibrosis (IPF), which again suggests that many TG2 substrates are likely involved in IPF pathogenesis. They further analyzed the data with CytoHubba and found that the ER stress and PPAR pathways were significantly enhanced in the bleomycin-treated WT mice. Lastly, they validated the proteomic data by evaluating the level of biotinylated pentylamine (BPA) incorporation into some known and newly found TG2 substrates in the lung sections from bleomycin-injured WT and TGKO mice.

This is the first time that proteomics data have been used to systematically identify TG2's substrate proteins in lung fibrosis, but the study also has limitations, some of which can be attributed to the shortcomings of the methods. First, the method has the advantage of being able to identify pepT26 or pentylamine *in situ* binding substrates in a TG2-dependent manner. However, this is only half of the story as



Supported by National Heart, Lung, and Blood Institute (grant HL135830) (G.L.), National Institute on Aging (grant AG050567) (Y.Y.S.), National Heart, Lung, and Blood Institute (grant HL151702) (Y.Y.S.), and United States Department of Defense (grant W81XWH-20-1-0226) (G.L.).

Originally Published in Press as DOI: 10.1165/rcmb.2021-0209ED on July 22, 2021

the method seems unable to identify the other side of the isopeptide bonds simultaneously (i.e., the glutamine donor polypeptides or proteins [in case of pepT26] or the lysine donor polypeptides or proteins [in case of pentylamine]). Either the current method needs to be improved or a validation of the crosslinking formation among the identified substrates by the two methods needs to be performed. Second, although this study has identified a number of TG2 substrate proteins, it is premature to conclude that any of these substrates may account for the profibrotic activity of TG2 in tissue fibrosis. Furthermore, even if a substrate is known to be involved in the disease mechanism, it is unclear how the indicated isopeptide bond affects its function or activity. Without this knowledge, one cannot truly appreciate the significance of these novel TG2 substrates in the disease initiation and progression. Nevertheless, this study represents an early and meaningful step in the right direction by expanding our perspective on the ways that TG2, and perhaps other protein crosslinking enzymes, contribute to the pathogenesis of lung fibrosis. ■

**Author disclosures** are available with the text of this article at www.atsjournals.org.

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