

# Dose-Dependent Antifibrotic Effect of Chrysin on Regression of Liver Fibrosis: The Role in Extracellular Matrix Remodeling

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## Abstract

Liver fibrosis represents an overaccumulation of extracellular matrix (ECM). This study was designed to investigate the effect of chrysin on established ECM overproduction in carbon tetrachloride (CCl<sub>4</sub>) mouse liver fibrosis. Experimental fibrosis was induced by intraperitoneal injection of 2 mL/kg CCl<sub>4</sub> twice a week, for 7 weeks. Mice were orally treated with 3 doses of chrysin (5,7-dihydroxyflavone). For the assessment of the spontaneous reversion of fibrosis, CCl<sub>4</sub>-treated mice were investigated after 2 weeks of recovery time. Silymarin was used as a standard of liver protection. In fibrotic livers, the results showed the upregulation of collagen I (Col I) and tissue inhibitors of metalloproteinase I (TIMP-I) and modulation of matrix metalloproteinases (MMPs), which led to an altered ECM enriched in Col, confirmed as well by electron microscopy investigations. Treatment with chrysin significantly reduced ultrastructural changes, downregulated Col I, and restored TIMP-I/MMP balance, whereas in the group observed for the spontaneous regression of fibrosis, they remained in the same pattern with fibrotic livers. In this study, we have shown chrysin efficacy to attenuate dose-dependent CCl<sub>4</sub>-stimulated liver ECM accumulation by regulation of MMP/TIMP imbalance and inhibition of Col production. We have shown the dose-dependent chrysin efficiency in attenuation of CCl<sub>4</sub>-induced liver ECM accumulation by regulation of MMP/TIMP imbalance and inhibition of Col production. Our findings suggest that chrysin oral administration may introduce a new strategy for treating liver fibrosis in humans.

## Keywords

liver fibrosis, chrysin, ECM, MMPs, TIMP-I, collagen

## Introduction

Hepatic fibrosis is a common wound healing response to chronic liver injury,<sup>1</sup> involving extra deposition of extracellular matrix (ECM) proteins.<sup>2,3</sup> The fibrotic ECM is composed of collagens, especially type I and III collagens,<sup>2</sup> structural glycoproteins, proteoglycans, and hyaluronan.<sup>4,5</sup> In the liver, activated hepatic stellate cells (HSCs) and myofibroblast (MF) with overlapping phenotypes deposit the majority of the fibrotic ECM. The main scar proteins produced by these fibrogenic effector cells are collagens, especially collagen type I, but several other proteins play a role in fibrotic matrix organization.<sup>6</sup>

Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) are the main regulators of ECM turnover in hepatic fibrosis.<sup>7</sup> The liver injury disturbs the TIMP/MMP balance and overexpresses TIMPs, contributing to ECM deposition and fibrosis progress.<sup>8</sup>

Matrix metalloproteinases are calcium-dependent zinc-containing endoproteinases, produced by connective and

inflammatory cells.<sup>9</sup> So far, 23 MMPs have been discovered in humans,<sup>10</sup> from which MMP-1, MMP-2, MMP-3, MMP-9, MMP-11, and MMP-13 are constitutively expressed in normal livers<sup>7</sup> by parenchymal cells such as hepatocytes or nonparenchymal cells such as HSCs, Kupffer cells, neutrophils, and

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recruited hepatic macrophages.<sup>9</sup> Matrix metalloproteinases are involved in ECM remodeling under normal physiological and pathologic conditions.<sup>11,12</sup> Matrix metalloproteinases degrade components of the ECM and numerous nonmatrix proteins.<sup>13</sup> Dysregulation of MMP activity often results in tissue damage and functional alterations.

Tissue inhibitors of metalloproteinases are a family of physiological inhibitors (TIMP 1-4) capable of regulating proteolytic activities of MMPs in tissues.<sup>14,15</sup> Tissue inhibitors of metalloproteinase 1 and 2 play a key role in the progression of hepatic fibrosis,<sup>2,16</sup> especially through inhibition of MMPs and antiapoptotic effect on HSCs.<sup>17</sup>

Several studies have been conducted to find natural bioactive compounds, such as luteolin, morin, quercetin, baicalein, or chlorogenic acid, capable of preventing retard or reverse liver fibrosis progression.<sup>18</sup> Chrysin (5,7-dihydroxy-2-phenyl-4*H*-chromen-4-one; 5,7-dihydroxyflavone) belongs to flavonoids class that has 15-carbon skeleton natural polyphenolic compounds.<sup>19</sup> It is present in propolis, honey,<sup>20</sup> many fruits,<sup>21,22</sup> plant extracts,<sup>23,24</sup> and even mushrooms.<sup>25</sup>

Previous studies have indicated that chrysin has several pharmacological activities, such as antioxidant,<sup>26-30</sup> antihypercholesterolemic,<sup>31</sup> antidiabetogenic,<sup>32</sup> and antitumor<sup>27,33,34</sup> activity. In addition, chrysin possesses anti-inflammatory effects via blocking histamine release and pro-inflammatory cytokine expression<sup>35,36</sup> and also protects liver from chemotherapeutic drugs and other hepatotoxic agents.<sup>37</sup> In a previous study, we demonstrated that chrysin has the efficacy to inhibit HSC activation and proliferation through transforming growth factor beta/Smad (TGF- $\beta$ 1/Smad) pathway.<sup>38</sup>

In the present study, we investigated the effects of chrysin on ECM modulation in CCl<sub>4</sub>-stimulated liver fibrotic mice. We focused our analysis on messenger RNA (mRNA) collagen expression and collagen liver distribution by histopathology and electron microscopy investigations. Furthermore, we evaluated the antifibrotic potential of chrysin by its capacity to alleviate the MMP/TIMP imbalance induced by CCl<sub>4</sub> chronic administration.

## Materials and Methods

### Materials

Chrysin 97%, silymarin (Sy) 98%, and carboxymethyl cellulose (CMC) were purchased from Sigma Aldrich Chemie GmbH (Munich, Germany). Primers were synthesized by Eurogentec (Liege, Belgium).

### Animals and Treatment

CD1 male mice from our animal facility were divided into 7 groups (n = 10), as shown in Figure 1. The mice were fed with a standard rodent diet and were maintained at 12-hour light/dark cycle at constant temperature and humidity. All experimental procedures were approved by the Ethical Research Committee of "Vasile Goldis" Western University of Arad.

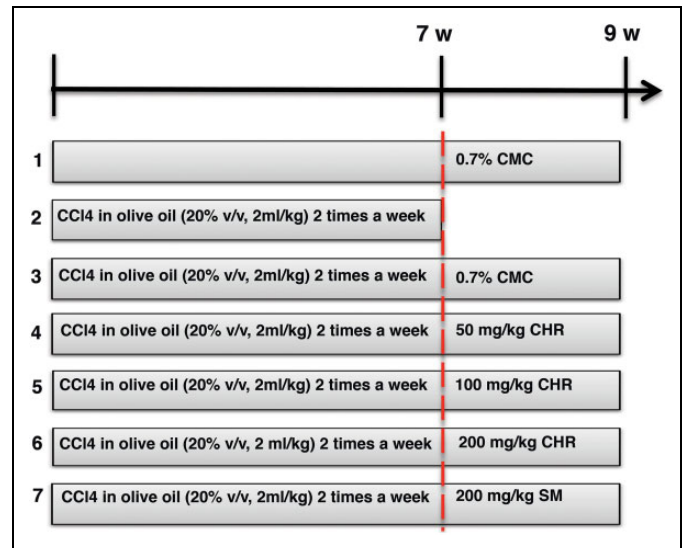


Figure 1. Schematic diagram of the experimental protocol.

Liver fibrosis was induced by intraperitoneal injection of CCl<sub>4</sub> dissolved in olive oil (20% vol/vol, 2 mL/kg), twice a week for 7 weeks, and mice were euthanatized 2 days after the last injection for liver fibrosis confirmation (group 2). In order to evaluate the spontaneous resolution of hepatic fibrosis, we kept mice for additional 2 weeks without any treatment (group 3). Groups 4, 5, and 6 received orally 50, 100, or 200 mg/kg chrysin for 14 days at the end of the 7 weeks of CCl<sub>4</sub> administration. For gavage administration, chrysin powder was dissolved in 0.7% CMC. Control animals (group 1) and animals in groups 2 and 3 were given orally an equal volume of 0.7% CMC solution for the last 2 weeks of experimental protocol. Silymarin in 0.7% CMC at 200 mg/kg dose was used as positive control (group 7).

### Histopathological Examination

The liver samples were embedded in paraffin, cut into 5- $\mu$ m-thick sections, and stained with Fouchet van Gieson according to the protocol provided with the Bio-Optica (Milano, Italy) staining kit. The criteria used for scoring fibrosis degree were evaluated on trichrome slides, modified after Wang et al<sup>39</sup>: 0, no obvious fibrosis (no collagen fibers); 1, collagen fibers present; 2, mild fibrosis (few collagen fibers extending without formation of compartments); 3, moderate fibrosis (collagen fibers with formation of "pseudo leaves"); 4, severe fibrosis (many collagen fibers with thickening of partial compartments and formation of "pseudolobes").

### Transmission Electron Microscopy

For transmission electron microscopy, the glutaraldehyde-fixed liver samples were washed with 0.1 M phosphate buffer and postfixed in 2% osmic acid (Sigma-Aldrich, St Louis, Missouri) in 0.15 M phosphate buffer (Sigma-Aldrich). Dehydration was performed in acetone and embedded in the epoxy

**Table 1.** Primers sequences used to identify EMC liver markers by RT-qPCR.

Target	Sense	Antisense
COL I	5'CAGCCGCTTCACCTACAGC 3'	5'TTTTGTATTCAATCACTGTCTTGCC 3'
TIMP-1	5'GGTGTGCACAGTGTTTCCCTGTTT 3'	5'TCCGTCCACAACAGTGAGTGTC A 3'
MMP-1	5'-GCAGCGTCAAGTTAACTGGAA-3'	5'-AACTACATTTAGGGGAGAGGTGT-3'
MMP-2	5'CAG GGA ATG AGT ACT GGG TCT ATT 3'	5' ACT CCA GTT AAA GGC AGC ATC TAC 3'
MMP-3	5'ACCAACCTATTCCTGGTTGCTGCT 3'	5'ATGGAACGGGACAAGTCTGTGGA 3'
MMP-9	5'GGACCCGAAGCGGACATTG 3'	5' CGTCGTCGAAATGGGCATCT 3'

Abbreviations: COL I, collagen I; MMP, matrix metalloproteinase; TIMP, tissue inhibitors of metalloproteinase.

embedding resin (Epon 812). Sections of 60 nm were made on Leica EM UC7 ultramicrotome (Leica Microsystems GmbH, Wetzlar, Germany) and analyzed with a Tecnai 12 Biotwin transmission electron microscope (FEI Company, Hillsboro, Oregon).

### Real-Time Polymerase Chain Reaction Analysis

Messenger RNA expressions of collagen I (Col I), TIMP-1, MMP-1, MMP-2, MMP-3, and MMP-9 were determined using real-time quantitative polymerase chain reaction (qPCR). Liver samples were collected in RNA lysis solution (Thermo Scientific, Vilnius, Lithuania). Total RNA was isolated using SV Total RNA Isolation Kit (Promega, Madison, Wisconsin) according to the manufacturer's protocol. The quantity and quality of purified RNA was assessed using a NanoDrop 8000 spectrophotometer (Thermo Scientific, Wilmington, Delaware), then reverse transcribed to corresponding complementary DNA (cDNA) using first-strand cDNA synthesis kit (Thermo Scientific).

Conditions for the reverse transcriptase reaction were 25°C for 5 minutes, 37°C for 60 minutes, and 70°C for 5 minutes. Real-time PCR was performed using Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific) with Mx3000P real-time PCR system (Agilent, Santa Clara, California). All samples were run in triplicate.

The sequences of the primers (synthesized by Eurogentec) used for Col I, TIMP-1, and MMP-1, 2, 3, 9 mRNA detection are presented in Table 1. Messenger RNA levels of target genes were normalized to the levels of glyceraldehyde 3 phosphate dehydrogenase, which was used as reference gene and assessed under the same experimental protocol. Relative expression changes were determined using the  $2^{-\Delta\Delta C(T)}$  method.<sup>40</sup>

### Statistical Analysis

Experimental data were statistically evaluated using GraphPad Prism 3.03 software (GraphPad Software, Inc, La Jolla, California) and one-way analysis of variance, followed by a Bonferroni test.  $P < .05$  was considered to indicate a statistically significant difference.

## Results

### Effect of Chrysin on Liver Collagen Accumulation

To observe the antifibrotic effects of chrysin and explore the underlying mechanisms, we first evaluate scoring fibrosis degree by collagen distribution, using Fouchet van Gieson staining. As shown in Figure 2A, the fibrotic livers showed typical characteristics of fibrosis (red triangles), and the fibrosis score increased significantly compared to the control ( $P < .001$ ), as confirmed by electron microscopy (Figure 2B). After treatment with chrysin, the fibrotic scores were significantly reduced in a dose-dependent manner. The Col I mRNA expression provided the same pattern (Figure 2D).

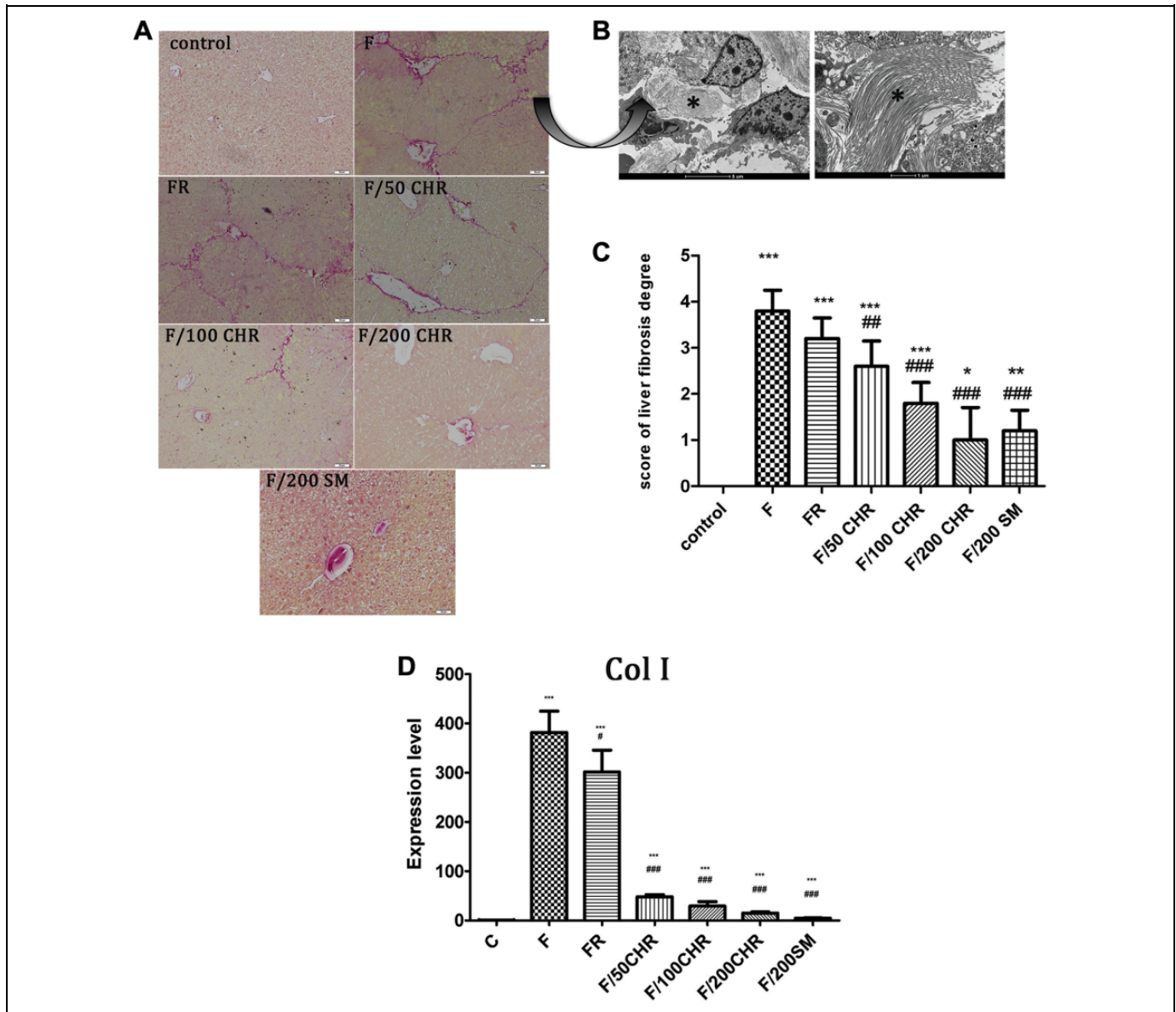
The collagen deposition was confirmed by electron microscopy, as shown in Figure 3. Electron microscopy micrographs of fibrotic group (F) highlights dense bundle of collagen fibers that proliferate in the parenchyma, space of Disse, and between swollen profiles of a sinusoid endothelial cell. Microvilli are not present on the surface of the adjacent hepatocyte. The ultrastructure of livers was alleviated on chrysin-treated liver in a dose-dependent manner.

### Effects of Chrysin on TIMP-1, MMP-2, MMP-3, and MMP-9 mRNA Expressions

Significant increase in TIMP-1, MMP-2, MMP-3, and MMP-9 mRNA expressions was detected in CCl<sub>4</sub>-induced liver fibrosis in mice, compared to control (Figure 4). With 14 days of daily chrysin administration, TIMP-1, MMP-2, MMP-3, and MMP-9 mRNA downregulated significantly ( $P < .001$ ), while MMP-1 mRNA upregulated significantly compared to fibrotic group ( $P < .001$ ) in a dose-dependent manner. The protective response to Sy was almost similar to 200 mg/kg of chrysin treatment.

## Discussion

Liver fibrosis occurs due to a dynamic wound healing response to hepatocellular damage and is accompanied by increased deposition of ECM in the perisinusoidal and periportal spaces.<sup>41</sup> Activated HSCs are the key factor involved in the collagen production,<sup>42</sup> due to phenotypical transformation into  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA)-positive MF-like cells, and increase their expression of fibrillar collagen and MMPs, as well as TIMPs.



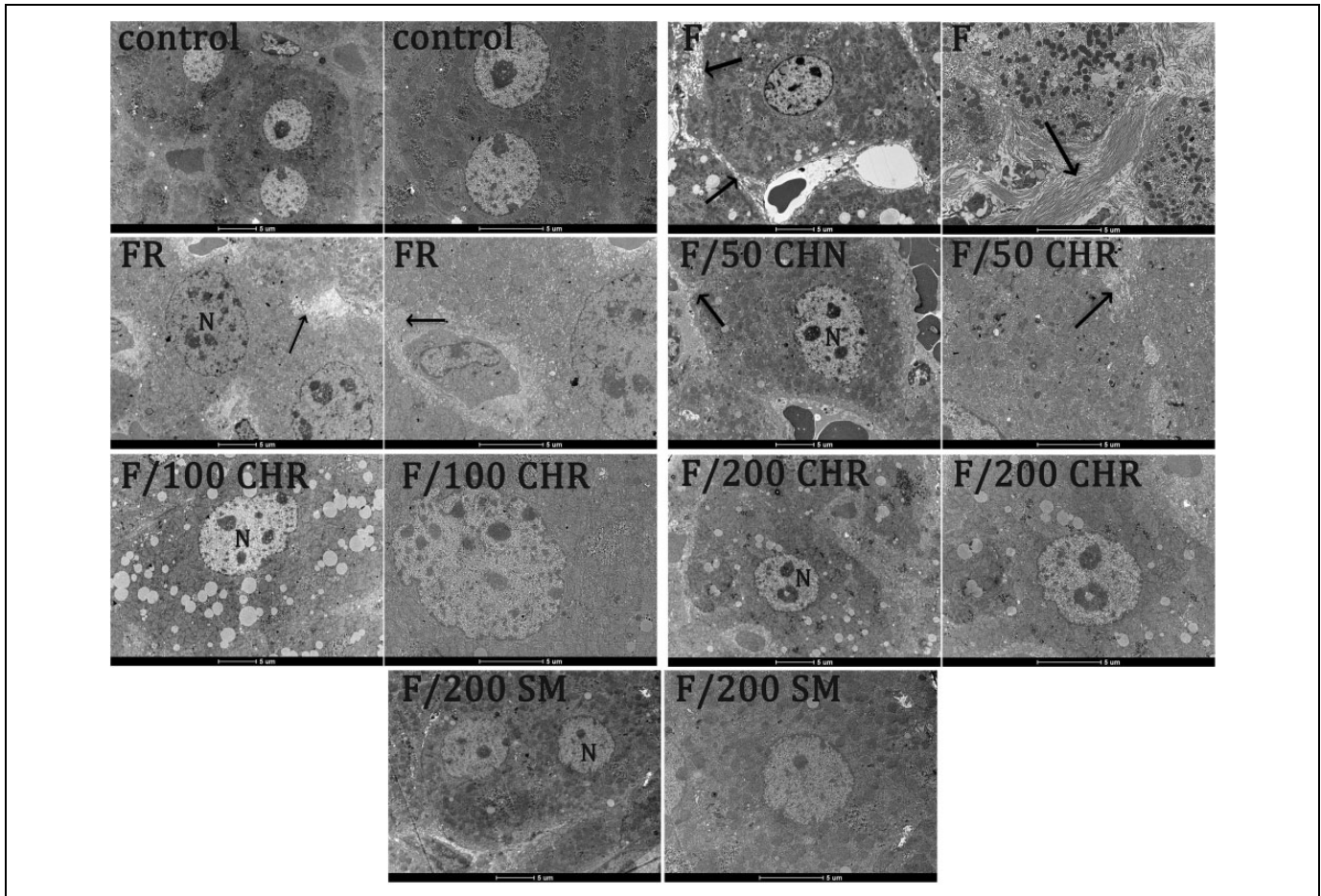
**Figure 2.** Chrysin inhibited extensive collagen deposition in liver tissue of tested mice. A, Fouchet van Gieson staining. Magnification,  $\times 20$ . B, Collagen fibers (\*) in F Group. C, The scores of the degree of liver fibrosis. D, Messenger RNA levels of collagen I. Significance levels are marked as follows: \*\*\* $P < .001$  compared to control; \*\* $P < .01$  compared to control; \* $P < .05$  compared to control; #### $P < .001$  compared to fibrotic group; \*\* $P < .01$  compared to fibrotic group. C indicates control group; F,  $\text{CCl}_4$  group; FR,  $\text{CCl}_4$  control group; F/50 CHR,  $\text{CCl}_4$  and 50 mg/kg chrysin; F/100 CHR,  $\text{CCl}_4$  and 100 mg/kg chrysin; F/200 CHR,  $\text{CCl}_4$  and 200 mg/kg chrysin; F/200 SM,  $\text{CCl}_4$  and 200 mg/kg silymarin.

In our previous study, we characterized that inhibition of TGF- $\beta$ 1/Smad signaling pathway plays an essential role in the antifibrotic effect of chrysin.<sup>38</sup> In this article, we deepened the effects of chrysin in  $\text{CCl}_4$ -induced liver injury in mice by highlighting the significant alleviation of liver fibrosis and ECM accumulation via TIMP/MMP rebalance and collagen production downregulation.

Matrix metalloproteinases are produced by activated HSCs and catalyze proteolysis, while TIMPs regulate ECM homeostasis by binding to a specific MMP and preventing its activity.<sup>43</sup> Matrix metalloproteinase modulation and, thus, fibrolysis

might be sufficient to shift the balance between fibrogenesis and fibrolysis toward fibrosis resolution.

The TIMP1 expression and secretion is strongly linked to HSC activation, related to the numbers of activated cells and profibrotic activity, and remains at high levels during progressive fibrosis.<sup>44</sup> The chronic administration of  $\text{CCl}_4$  for 7 weeks stimulates HSCs to secrete TIMP-1. During fibrosis resolution induced by chrysin administration, the gene expression of TIMP-1 was rapidly declined in a dose-dependent manner, tipping the overall TIMP/MMP balance, resulting in increased matrix degrading activity and net degradation of scar tissue,<sup>45</sup>



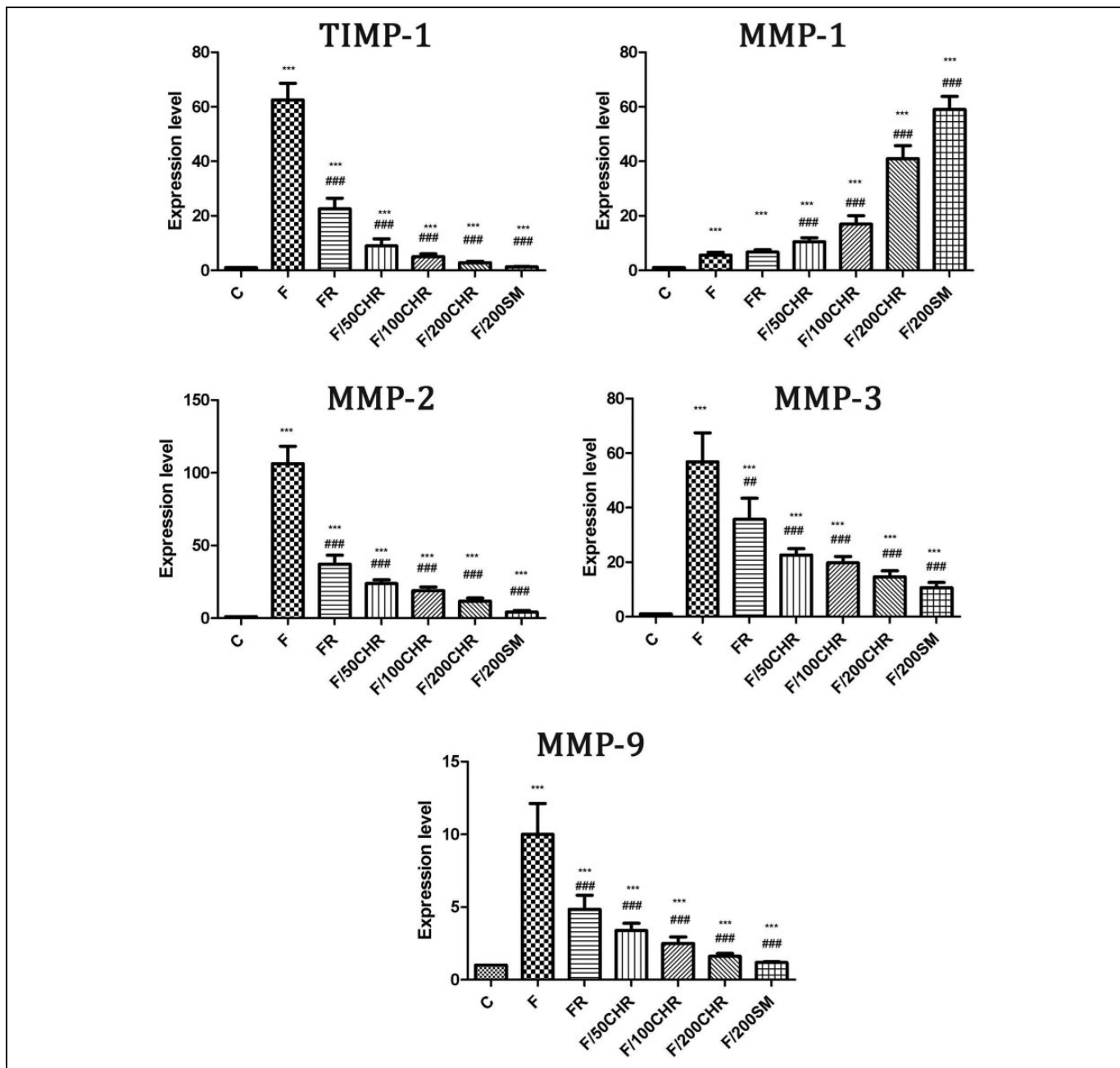
**Figure 3.** Collagen deposition in experimental livers by electron microscopy. Collagen fibers (arrow). C indicates control group; F, CCl<sub>4</sub> group; FR, CCl<sub>4</sub> control group; F/50 CHR, CCl<sub>4</sub> and 50 mg/kg chrysin; F/100 CHR, CCl<sub>4</sub> and 100 mg/kg chrysin; F/200 CHR, CCl<sub>4</sub> and 200 mg/kg chrysin; F/200 SM, CCl<sub>4</sub> and 200 mg/kg Silymarin; N, nucleus.

as shown in Figure 2. Therefore, it is possible that chrysin regulates the ECM balance via TIMP/MMP components and inhibits the activation and proliferation of HSCs, confirmed by our previous findings where chrysin induced  $\alpha$ -SMA and TGF- $\beta$ 1/Smad downregulation.<sup>38</sup> Similar results were obtained with other flavonoids, such as quercetin,<sup>39</sup> isoorientin,<sup>46</sup> and Sy,<sup>47</sup> in CCl<sub>4</sub>-induced liver fibrosis or secondary biliary fibrosis models. As a consequence, chrysin treatment induced downregulation of Col I at transcriptional and translational levels in a dose-dependent manner.

Matrix metalloproteinase 1, also known as interstitial collagenase or fibroblast collagenase,<sup>13</sup> is the main protease that can degrade type I collagen, which usually represents the fibrotic scaffold and >50% of the scar protein.<sup>48</sup> This study found that chrysin can upregulate MMP-1 mRNA expression and further stimulate cleavage of the native fibrillar collagens, especially Col I by regulating the ECM balance via TIMP-1/MMP-1 components. Matrix metalloproteinase 1 downregulated in established CCl<sub>4</sub>-liver fibrosis, is expressed again during the resolution process, and may act through ECM degradation as well as by induction of HSC apoptosis,<sup>49</sup> confirmed by our previous findings.<sup>38</sup>

There is a growing evidence that MMP-2 and MMP-9 (gelatinases) play an important role in the pathogenesis of numerous disorders associated with ECM remodeling, including liver cirrhosis.<sup>50</sup> Matrix metalloproteinase 2 is synthesized by active HSCs.<sup>51</sup> Matrix metalloproteinase 2 is involved in remodeling of basement membranes in early phases of liver fibrosis by replacement of normal subendothelial matrix with interstitial collagen and further accelerates HSC activation.<sup>52</sup> In our study, we found that MMP-2 mRNA expression remained at a higher level after 7 weeks of CCl<sub>4</sub> administration, which is in agreement with other findings.<sup>53</sup> Our data showed that chrysin completely abrogates MMP-2 upregulation induced by CCl<sub>4</sub> administration. Recent studies showed that inhibition of MMP-2 activity or blockade of MMP-2 synthesis with other natural compounds, such as betaine<sup>54</sup> or morin,<sup>55</sup> might effectively prevent HSC activation and proliferation and collagen accumulation. Furthermore, MMP-3 and MMP-9 also contribute to HSC activation.<sup>56,57</sup> Our results demonstrated chrysin's potential to modulate the gene expression of TIMP-1, MMP-2, MMP-3, and MMP-9 in liver tissue. The ability of chrysin to suppress TIMP-1, MMP-2, MMP-3, and MMP-9 expressions





**Figure 4.** Messenger RNA levels of TIMP-1, MMP-1, MMP-2, MMP-3, and MMP-9 in liver tissue of tested mice. Significance levels are marked as follows: \*\*\* $P < .001$  compared with control; ### $P < .01$  and #### $P < .001$  compared with CCl<sub>4</sub> fibrotic livers. C indicates control group; F, CCl<sub>4</sub> group; FR, CCl<sub>4</sub> control group; F/50 CHR, CCl<sub>4</sub> and 50 mg/kg chrysin; F/100 CHR, CCl<sub>4</sub> and 100 mg/kg chrysin; F/200 CHR, CCl<sub>4</sub> and 200 mg/kg chrysin; F/200 SM, CCl<sub>4</sub> and 200 mg/kg silymarin; MMP, matrix metalloproteinase; TIMP, tissue inhibitors of metalloproteinase.

helps to explain its antifibrogenic effect in the CCl<sub>4</sub>-induced liver fibrosis model in mice.

## Conclusion

This study revealed that chrysin possesses a therapeutic effect on CCl<sub>4</sub>-induced liver fibrosis. The antifibrotic effect of chrysin is associated with its ability to modulate ECM by TIMP/MMP rebalance and decrease collagen deposition in a

dose-dependent manner. Looking forward to clinical application, this insight may highlight a possible new therapeutic strategy against liver fibrosis in humans. Further studies have to be conducted on human beings before using this new strategy.


## Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## References

1. Friedman SL. Liver fibrosis—from bench to bedside. *J Hepatol.* 2003;38(suppl 1):38-53.
2. Xu GF, Li PT, Wang XY, et al. Dynamic changes in the expression of matrix metalloproteinases and their inhibitors, TIMPS, during hepatic fibrosis induced by alcohol in rats. *World J Gastroenterol.* 2004;10(24):3621-3627.
3. Benyon RC, Iredale JP. Is liver fibrosis reversible? *Gut.* 2000;46(4):443-446.
4. Karsdal MA, Manon-Jensen T, Genovese F, et al. Novel insights into the function and dynamics of extracellular matrix in liver fibrosis. *Am J Physiol Gastrointest Liver Physiol.* 2015;308(10):G807-G830.
5. Mouw JK, Ou G, Weaver VM. Extracellular matrix assembly: a multiscale deconstruction. *Nat Rev Mol Cell Biol.* 2014;15(12):771-785.
6. Schuppan D, Ruehl M, Somasundaram R, Hahn EG. Matrix as a modulator of hepatic fibrogenesis. *Semin Liver Dis.* 2001;21(3):351-372.
7. Duarte S, Baber J, Fujii T, Coito AJ. Matrix metalloproteinases in liver injury, repair and fibrosis. *Matrix Biol.* 2015;44-46:147-56.
8. Iredale JP, Thompson A, Henderson NC. Extracellular matrix degradation in liver fibrosis: biochemistry and regulation. *Biochim Biophys Acta.* 2013;1832(7):876-883.
9. Iredale JP. Models of liver fibrosis: exploring the dynamic nature of inflammation and repair in a solid organ. *J Clin Invest.* 2007;117(3):539-548.
10. Puente XS, Sanchez LM, Overall CM, Lopez-Otin C. Human and mouse proteases: a comparative genomic approach. *Nat Rev Genet.* 2003;4(7):544-558.
11. Bonnans C, Chou J, Werb Z. Remodelling the extracellular matrix in development and disease. *Nat Rev Mol Cell Biol.* 2014;15(12):786-801.
12. Klein T, Bischoff R. Physiology and pathophysiology of matrix metalloproteinases. *Amino Acids.* 2011;41(2):271-290.
13. Naim A, Pan Q, Baig MS. Matrix metalloproteinases (MMPs) in liver diseases. *J Clin Exp Hepatol.* 2017;7(4):367-372.
14. Kessenbrock K, Plaks V, Werb Z. Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell.* 2010;141(1):52-67.
15. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res.* 2003;92(8):827-839.
16. Nie QH, Zhang YF, Xie YM, et al. Correlation between TIMP-1 expression liver fibrosis in two rat liver fibrosis models. *World J Gastroenterol.* 2006;12(19):3044-3049.
17. Kawada N. Human hepatic stellate cells are resistant to apoptosis: implications for human fibrogenic liver disease. *Gut.* 2006;55(8):1073-1074.
18. Hermenean A, Smeu C, Gharbia S, Krizbai IA, Ardelean A. Plant-derived biomolecules and drug delivery systems in the treatment of liver and kidney diseases. *Curr Pharm Des.* 2016;22(35):5415-5441.
19. Samarghandian S, Farkhondeh T, Azimi-Nezhad M. Protective effects of chrysin against drugs and toxic agents. *Dose Response.* 2017;15(2):1559325817711782. doi:10.1177/1559325817711782.
20. Farkhondeh T, Samarghandia S, Azimin-Nezha M, Samin F. Effect of chrysin on nociception in formalin test and serum levels of noradrenalin and corticosterone in rats. *Int J Clin Exp Med.* 2015;8(2):2465-2470.
21. Song WJ, Li YH, Wang JG, Li ZY, Zhang JQ. Characterization of nucleobases and nucleosides in the fruit of *Alpinia oxyphylla* collected from different cultivation regions. *Drug Test Anal.* 2014;6(3):239-245.
22. Qing ZJ, Yong W, Hui LY, et al. Two new natural products from the fruits of *Alpinia oxyphylla* with inhibitory effects on nitric oxide production in lipopolysaccharide activated RAW264.7 macrophage cells. *Arch Pharm Res.* 2012;35(12):2143-2146.
23. Mehri S, Karami HV, Hassani FV, Hosseinzadeh H. Chrysin reduced acrylamide-induced neurotoxicity in both in vitro and in vivo assessments. *Iran Biomed J.* 2014;18(2):101-106.
24. Siess MH, Le Bon AM, Canivenc Lavier MC, et al. Flavonoids of honey and propolis: characterization and effects on hepatic drug metabolizing enzymes and benzo[a]pyrene DNA binding in rats. *J Agric Food Chem.* 1996;44(8):2297-2301.
25. Anandhi R, Annadurai T, Anitha TS, et al. Antihypercholesterolemic and antioxidative effects of an extract of the oyster mushroom, *Pleurotus ostreatus*, and its major constituent, chrysin, in Triton WR-1339-induced hypercholesterolemic rats. *J Physiol Biochem.* 2013;69(2):313-323.
26. Ali N, Rashid S, Nafees S, Hasan SK, Sultana S. Beneficial effects of chrysin against methotrexate-induced hepatotoxicity via attenuation of oxidative stress and apoptosis. *Mol Cell Biochem.* 2014;385(1-2):215-223.
27. Brechbuhl HM, Kachadourian R, Min E, Chan D, Day BJ. Chrysin enhances doxorubicin-induced cytotoxicity in human lung epithelial cancer cell lines: the role of glutathione. *Toxicol Appl Pharmacol.* 2012;258(1):1-9.
28. Anand KV, Anandhi R, Pakkiyaraj M, Geraldine P. Protective effect of chrysin on carbon tetrachloride (CCl<sub>4</sub>) induced tissue injury in male Wistar rats. *Toxicol Ind Health.* 2011;27(10):923-933.
29. Ciftci O, Ozdemir I. Protective effects of quercetin and chrysin against 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induced oxidative stress, body wasting and altered cytokine productions in rats. *Immunopharmacol Immunotoxicol.* 2011;33(3):504-508.
30. Pushpavalli G, Kalaiarasi P, Veeramani C, Pugalendi KV. Effect of chrysin on hepatoprotective and antioxidant status in D-galactosamine-induced hepatitis in rats. *Eur J Pharmacol.* 2010a;631(1-3):36-41.

31. Anandhi R, Thomas PA, Geraldine P. Evaluation of the anti-atherogenic potential of chrysin in Wistar rats. *Mol Cell Biochem.* 2014;385(1-2):103-113.
32. Samarghandian S, Azimin-Nezhad M, Samini F, Farkhondeh T. Chrysin treatment improves diabetes and its complications in streptozotocin-induced diabetic rat. *Can J Physiol Pharmacol.* 2016;94(4):388-393.
33. Khan MS, Devaraj H, Devaraj N. Chrysin abrogates early hepatocarcinogenesis and induces apoptosis in *N*-nitrosodiethylamine-induced preneoplastic nodules in rats. *Toxicol Appl Pharmacol.* 2011;251(1):85-94.
34. Phan TA, Yu XM, Kunnimalaiyaan M, Chen H. Antiproliferative effect of chrysin on anaplastic thyroid cancer. *J Surg Res.* 2011;170(1):84-88.
35. Bae Y, Lee S, Kim SH. Chrysin suppresses mast cell-mediated allergic inflammation: involvement of calcium, caspase-1 and nuclear factor- $\kappa$ B. *Toxicol Appl Pharmacol.* 2011;254(1):56-64.
36. Samarghandian S, Afshari JT, Davoodi S. Chrysin reduces proliferation and induces apoptosis in the human prostate cancer cell line pc-3. *Clinics (Sao Paulo)*, 2011;66(6):1073-1079.
37. Pushpavalli G, Veeramani C, Pugalendi KV. Influence of chrysin on hepatic marker enzymes and lipid profile against D-galactosamine-induced hepatotoxicity rats. *Food Chem Toxicol.* 2010b;48(6):1654-1659.
38. Balta C, Herman H, Boldura OM, et al. Chrysin attenuates liver fibrosis and hepatic stellate cell activation through TGF $\beta$ /Smad signaling pathway. *Chem Biol Interact.* 2015;240:94-101.
39. Wang R, Zhang H, Wang Y, Song F, Yuan Y. Inhibitory effects of quercetin on the progression of liver fibrosis through the regulation of NF- $\kappa$ B/I $\kappa$ B $\alpha$ , p38 MAPK, and Bcl-2/Bax signaling. *Int Immunopharmacol.* 2017;47:126-133.
40. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2 $\Delta\Delta$ C(T) Method. *Methods.* 2001;25(4):402-408.
41. Friedman SL. Hepatic fibrosis—overview. *Toxicology.* 2008;254(3):120-129.
42. Mallat A, Lotersztajn S. Cellular mechanisms of tissue fibrosis. 5. Novel insights into liver fibrosis. *Am J Physiol Cell Physiol.* 2013;305(8):C789-C799.
43. Hemmann S, Graf J, Roderfeld M, Roeb E. Expression of MMPs and TIMPs in liver fibrosis—a systematic review with special emphasis on anti-fibrotic strategies. *J Hepatol.* 2007;46(5):955-975.
44. Iredale JP, Benyon RC, Arthur MJ, et al. Tissue inhibitor of metalloproteinase-1 messenger RNA expression is enhanced relative to interstitial collagenase messenger RNA in experimental liver injury and fibrosis. *Hepatology.* 1996;24(1):176-184.
45. Issa R, Zhou X, Constandinou CM, et al. Spontaneous recovery from micronodular cirrhosis: evidence for incomplete resolution associated with matrix cross-linking. *Gastroenterology.* 2004;126(7):1795-1808.
46. Lin X, Chen Y, Lv S, et al. *Gypsophila elegans* isoorientin attenuates CCl<sub>4</sub>-induced hepatic fibrosis in rats via modulation of Nf- $\kappa$ B and TGF $\beta$ 1/Smad signaling pathways. *Int Immunopharmacol.* 2015;28(1):305-312.
47. Jia J-D, Bauer M, Cho JJ, et al. Antifibrotic effect of silymarin in rat secondary biliary fibrosis is mediated by downregulation of procollagen  $\alpha$ 1(I) and TIMP-1. *J Hepatol.* 2001;35(3):392-398.
48. Molokanova O, Schönig K, Weng S-Y, et al. Inducible knock-down of procollagen I protects mice from liver fibrosis and leads to dysregulated matrix genes and attenuated inflammation. *Matrix Biol.* 2017;66:34-49. doi:10.1016/j.matbio. 2017.11.002.
49. Han IP. Matrix metalloproteinases, the pros and cons, in liver fibrosis. *J Gastroenterol. Hepatol.* 2009;21(3):S88-S91.
50. Kurzepa J, Mdro A, Czechowska G, et al. Role of MMP-2 and MMP-9 and their natural inhibitors in liver fibrosis, chronic pancreatitis and non-specific inflammatory bowel diseases. *Hepatobiliary Pancreat Dis Int.* 2014;13(6):570-579.
51. Arriola Benitez PC, Rey Serantes D, Herrmann CK, et al. The effector protein BPE005 from *Brucella abortus* induces collagen deposition and matrix metalloproteinase 9 downmodulation via transforming growth factor  $\beta$ 1 in hepatic stellate cells. *Infect Immun.* 2015;84(2):598-606.
52. Li Y, Liu F, Ding F, Chen P, Tang M. Inhibition of liver fibrosis using vitamin A-coupled liposomes to deliver matrix metalloproteinase-2 siRNA in vitro. *Mol Med Rep.* 2015;12(3):3453-3461.
53. Cheung KF, Ye DW, Yang ZF, et al. Therapeutic efficacy of traditional Chinese medicine 319 recipe on hepatic fibrosis induced by carbon tetrachloride in rats. *J Ethnopharmacol.* 2009;124(1):142-150.
54. Bingül İ, Başaran-Küçükgergin C, Aydın AF, et al. Betaine treatment decreased oxidative stress, inflammation, and stellate cell activation in rats with alcoholic liver fibrosis. *Environ Toxicol Pharmacol.* 2016;45:170-178.
55. Perumal N, Perumal M, Halagowder D, Sivasithamparam N. Morin attenuates diethylnitrosamine-induced rat liver fibrosis and hepatic stellate cell activation by co-ordinated regulation of Hippo/Yap and TGF- $\beta$ 1/Smad signaling. *Biochimie.* 2017;140:10-19.
56. Roeb E. Matrix metalloproteinases and liver fibrosis (translational aspects). *Matrix Biol.* 2017;68-69:463-473. doi:10.1016/j.matbio. 2017.12.012.
57. Jackson PL, Xu X, Wilson L, et al. Human neutrophil elastase-mediated cleavage sites of MMP-9 and TIMP-1: implications to cystic fibrosis proteolytic dysfunction. *Mol Med.* 2010;16(5-6):159.