**Borthwick LA, Mann DA. Osteopontin and HMGB1: novel regulators of HSC activation. *Nature Reviews Gastroenterology & Hepatology* 2016, 13(6), 320-322.**

**Osteopontin and high mobility group box-1: Novel regulators of hepatic stellate cell activation**

Lee A Borthwick[1]* & Derek A Mann[1]

1. Tissue Fibrosis and Repair Group, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK, NE2 4HH.

*** Correspondence author contact information**

Dr Lee Borthwick
Fibrosis Research Group
Institute of Cellular Medicine
4th Floor, William Leech Building
Newcastle University
Newcastle upon Tyne
NE2 4HH
Tel: 0191 208 3886
Email: lee.borthwick@ncl.ac.uk

**Word count:** 1075

Liver fibrosis is a pathological process in which a pivotal event is the activation of non-parenchymal hepatic stellate cells (HSC) into scar-forming liver myofibroblasts in response to parenchymal liver cell damage[1]. The molecular mechanisms that cross-talk between damaged hepatocytes and non-parenchymal cells are therefore of significant interest towards discovering novel ways of modulating fibrosis progression in patients with chronic liver disease. Osteopontin (OPN) is a multi-functional protein and an important part of the extracellular matrix that has been demonstrated to play many important physiological and pathological functions in a wide range of tissues[2]. For example OPN functions in the regulation of the immune response[3], the modulation of cell proliferation, stimulation of angiogenesis, and regulates tumour cell invasion and migration in hepatocellular carcinoma[4 5].

A growing body of experimental data indicates that OPN also contributes to the injury response of the liver and in particular in the fibrogenic process. In previous work, Nieto and colleagues demonstrated that OPN deficient mice display enhanced hepatocyte proliferation and are protected from thioacetamide and bile duct ligation-induced liver injury, with impacts on the degree of ductular reaction and scarring. Mechanistically the authors showed effects of OPN on proliferation of hepatic progenitor cells and reported enhanced production of collagen-I by HSC *via* an OPN/TGF-β dependant pathway[6]. In agreement with these findings, Coombes *et al*. showed that neutralising osteopontin using two different approaches (aptamer and neutralising antibody) abrogated scarring in three distinct models of liver fibrosis (carbon tetrachloride ($CCl_4$), methionine-choline deficient diet and the 3,5,-diethoxycarbonyl-1,4-dihydrocollidine diet) by modulating TGF-β signalling[7]. Furthermore serum OPN has been identified as a potential prognostic index for reporting progression of hepatic fibrosis to cirrhosis and hepatocellular carcinoma[8].

In the latest study from the Nieto group, Arriazu *et al*, through a series of detailed experimental approaches, provide important new mechanistic insights into the fibrogenic actions of OPN that suggest the existence of both paracrine and autocrine pathways. Important to their thesis is their initial observation of enhanced co-expression, as well as cellular co-localisation, of OPN with an important signalling molecule; high mobility group box 1 protein (HMGB1). This close association between OPN and HMGB1 is described in hepatocytes and HSCs in human HCV-induced liver fibrosis and in experimental rodent liver fibrosis. To begin their experimental investigations, the authors confirmed that hepatic over-expression of OPN enhances $CCl_4$-induced fibrosis whereas genetic deletion of OPN was protective. A role for HMGB1 in hepatocytes was demonstrated by selective deletion *in vivo*, which resulted in partial protection from $CCl_4$-induced fibrosis. However, serum ALT

and AST activities were also lower in these mice possibly indicating an unexplored hepatoprotective role for HMGB1 upstream of fibrogenesis. To interrogate the fibrogenic relationship between OPN and HMGB1 the authors carried out a series of elegant co-culture experiments in which they show that soluble hepatocyte-derived OPN and HMGB1 stimulate collagen production by HSC. Moreover, OPN was discovered to operate upstream of HMGB1 by inducing its expression and release from hepatocytes. Extracellular HMGB1 acts as a ligand for a number of different pattern recognition receptors such as TLRs and RAGE[9]. The authors identify RAGE as the key mediator of HMGB1 effects on HSC by using lentiviral expression vectors to achieve shRNA knockdown of TLRs 2/4/9 and RAGE and then showing that only knockdown of RAGE impacted on HMGB1-induced collagen expression. Furthermore, they identify a signalling pathway downstream of RAGE involving activation of the intracellular signalling molecules PI3K and pAKT which in turn stimulate collagen-I gene expression. Returning to *in vivo*, the ability of a RAGE neutralising antibody to partially block liver fibrosis confirmed the involvement of RAGE. Taken together these experiments nicely describe a novel autocrine pathway triggered by OPN/HMGB1 interactions in hepatocytes that result in release of extracellular HMGB1 leading to stimulation of collagen expression by HSC *via* a RAGE/PI3K/pAKT dependent pathway (figure 1).

A further key observation was that addition of recombinant OPN (rOPN) to cultured HSC stimulated expression of HMGB1 and collagen, an effect that was found to be post-transcriptional. In addition rOPN stimulated translocation of HMGB1 from its usual nuclear location to the cytoplasm and this relocation correlated with collagen-I expression. Nuclear to cytoplasmic shuttling of HMGB1 is at least in-part controlled by acetyl-lysine modifications in the protein[10] and forced expression of a mutated version of HMGB1 lacking these acetyl-lysine residues blocked rOPN-induced collagen expression. Impressively the authors went on to describe the molecular events by which OPN cross-talks with the regulatory machinery of HMGB1 acetylation. Briefly, mass spectrometry biochemically confirmed that rOPN induces HMGB1 lysine acetylation in HSC and these modifications were associated with the selective reduction in histone deactylase 1 and 2 (HDAC1/2) expression mediated *via* NOX-derived ROS. The importance of this pathway was confirmed by showing that treatment of HSC with NOX inhibitors blocked rOPN-induced loss of HDAC1/2 expression and also inhibited rOPN-induced collagen expression (figure 1). As HSC express both OPN and HMGB1 in fibrotic liver these experiments highlight the potential for an autocrine route by which the two combine to provoke HSC to produce collagen.

Taken together the various investigational findings of this new study not only reinforce the pathophysiological relevance of OPN in liver fibrosis but illuminate HMGB1 as an important downstream mediator that orchestrates paracrine and autocrine fibrogenic actions of OPN. Additionally, a number of other molecular regulators are identified that may be exploited for drug discovery including RAGE, HDAC1/2, NOX, PI3K and pAKTs. However the study also raises a number of questions that need to be addressed if the OPN/HMGB1 axis is to be therapeutically manipulated. In particular, the relative importance of paracrine versus autocrine signalling is opaque. Notably the authors did not genetically target knockout of OPN or HMGB1 to HSC; the latter being possible *via* several available HSC-selective Cre drivers. The precise mechanisms by which HSC-expressed HMGB1 stimulates collagen expression is also unclear and firm experimental evidence for the requirement of HDAC1/2 should be investigated as these proteins have multiple roles with potential to impact on collagen expression, as indeed does NOX-derived ROS. Finally, once in the extracellular microenvironment OPN can be cleaved by proteases such as thrombin generating at least two different isoforms (OPN-R and OPN-L). Further there is an intracellular form known as iOPN and several alternative splice variants (OPN-a, b and c) have also been described[11]. OPN has important physiological functions vital to tissue homeostasis and is considered a difficult molecule for therapeutic manipulation, however if one or more isoforms are chiefly responsible for OPNs fibrogenic activities then selective targeting strategies may emerge.

**Figure legend**

**Figure 1 - Proposed mechanism of Osteopontin and HMGB-1 in liver fibrosis**

AC-acetylation, P-Phosphorylation.

**References**


1 Trautwein, C., Friedman, S. L., Schuppan, D. & Pinzani, M. Hepatic fibrosis: Concept to treatment. *J Hepatol* **62**, S15-24, doi:10.1016/j.jhep.2015.02.039 (2015).

2 Subraman, V., Thiyagarajan, M., Malathi, N. & Rajan, S. T. OPN -Revisited. *J Clin Diagn Res* **9**, ZE10-13, doi:10.7860/JCDR/2015/12872.6111 (2015).

3 Rittling, S. R. & Singh, R. Osteopontin in Immune-mediated Diseases. *J Dent Res* **94**, 1638-1645, doi:10.1177/0022034515605270 (2015).

4 Nagoshi, S. Osteopontin: Versatile modulator of liver diseases. *Hepatol Res* **44**, 22-30, doi:10.1111/hepr.12166 (2014).

5 Kale, S. *et al.* Osteopontin signaling upregulates cyclooxygenase-2 expression in tumor-associated macrophages leading to enhanced angiogenesis and melanoma growth via alpha9beta1 integrin. *Oncogene* **33**, 2295-2306, doi:10.1038/onc.2013.184 (2014).

6 Wang, X. *et al.* Osteopontin induces ductular reaction contributing to liver fibrosis. *Gut* **63**, 1805-1818, doi:10.1136/gutjnl-2013-306373 (2014).

7 Coombes, J. D. *et al.* Osteopontin neutralisation abrogates the liver progenitor cell response and fibrogenesis in mice. *Gut* **64**, 1120-1131, doi:10.1136/gutjnl-2013-306484 (2015).

8 Matsue, Y. *et al.* Serum osteopontin predicts degree of hepatic fibrosis and serves as a biomarker in patients with hepatitis C virus infection. *PLoS One* **10**, e0118744, doi:10.1371/journal.pone.0118744 (2015).

9 Li, L. C., Gao, J. & Li, J. Emerging role of HMGB1 in fibrotic diseases. *J Cell Mol Med* **18**, 2331-2339, doi:10.1111/jcmm.12419 (2014).

10 Bonaldi, T. *et al.* Monocytic cells hyperacetylate chromatin protein HMGB1 to redirect it towards secretion. *EMBO J* **22**, 5551-5560, doi:10.1093/emboj/cdg516 (2003).

11 Kazanecki, C. C., Uzwiak, D. J. & Denhardt, D. T. Control of osteopontin signaling and function by post-translational phosphorylation and protein folding. *J Cell Biochem* **102**, 912-924, doi:10.1002/jcb.21558 (2007).