HTRF and AlphaLISA assays bring clarity to extracellular matrix related fibrosis development

Abstract

Fibrotic disorders are a collection of complex pathologies that are thought to result from improperly monitored and excessive wound healing mechanisms. Unraveling the exact pathogenesis of those disorders is challenging as they involve the complex regulation of inflammation, but the understanding of their mechanisms is critical as organ fibrosis is a life threatening condition that can only be addressed with transplants. This note gathers publications that exemplify various applications of HTRF™ and AlphaLISA™ assays to advance fibrosis related research and shows how other researchers have harnessed these technologies to move their studies forward.

Introduction

Fibroblasts are specialized wound healing cells found across all organs and responsible for maintaining the structural integrity of tissues through regulation and care of the extracellular matrixes (ECM). To fulfill their role, they are equipped with a collection of receptors allowing them to receive inflammatory mediators and move toward injury sites. They are also capable of activation in response to infectious pathogens thanks to their set of Toll-Like Receptors (TLRs), which are innate immunity receptors. TLRs sensing and motility abilities allow them to identify potential sources of damages both from injuries and infection, and to perform their wound healing and scarring functions in both.

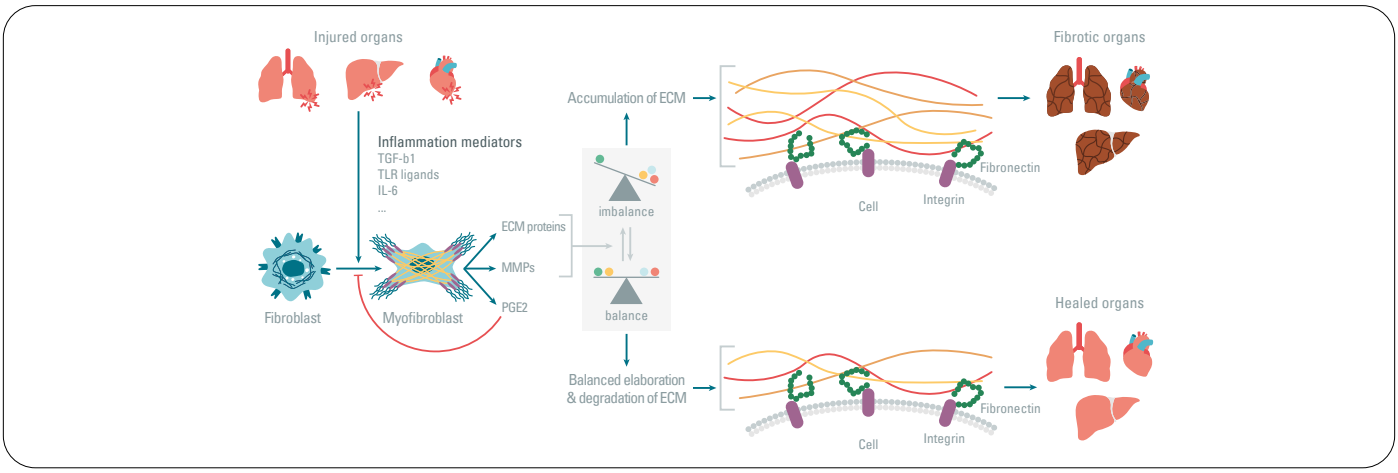

Figure 1: Example of fibroblasts involvement in fibrosis pathogenesis.

Fibroblasts are especially activated by growth factors expressed at injury sites such as TGF-β1, pro-inflammatory cytokines (IL-6, IL-1β) and TLR ligands. Upon activation and migration to the site, they proliferate and differentiate into active myofibroblast with increased secretory and contractility properties.

These cells promote wound healing via the secretion of ECM proteins such as collagens and matrix metalloproteinases (MMPs), which are respectively responsible for elaborating and degrading the extracellular matrix. Active fibroblasts are usually kept under control by the mediator PGE2, which inhibits their proliferation and prevents excessive secretion of ECM proteins. Adhesion of myofibroblasts and tissue cells to the matrix is made possible by the transmembrane integrin interaction with the extracellular fibronectin which bind the matrix. Finally, myofibroblast promote wound closure thanks to their contractility, which brings nearby cells closer together by pulling on the ECM matrix.

The elaboration and degradation of the ECM is a delicate balance under the regulation of all the inflammatory mediators involved in fibroblasts activity. In the case a fibrotic disorders, this balance is compromised and the resulting excess of ECM or lack of MMPs can lead to a disorganized accumulation of ECM which, in later stages, results in organ rigidity and fibrosis. For this reason, the ECM constitutive proteins, MMPs and fibroblasts activating and regulatory mediators such as TGF-β1 and PGE2 are hot spots of therapeutic research for fibrosis.

PGE2 activation in lung fibroblasts

Wright, W. et al. *Prostaglandins & Other Lipid Mediators. Volume 107, pages 4-12 (2013)*

Lung fibroblasts have been increasingly associated with idiopathic pulmonary fibrosis and current research has taken a special interest in lipid mediators of the prostanoid family as regulatory partners in the wound healing process that prevent escalation to fibrotic disorders. Among those, PGE2 is especially known for its fibroblast inhibitory potential which enables re-epithelialization. The regulation of PGE2 itself is under the control of the COX-1 and COX-2 enzymes.

In 2017, Wright et al. sought to better understand how COX enzymes regulate PGE2 levels in TLR stimulated fibroblasts. Using the HTRF PGE2 kit they monitored PGE2 release in different fibroblast models both murine and human, with different COX-1 and COX-2 expression levels. Their study included an additional layer of analysis by looking at the stimulation of different specific TLR types, using specific ligands to target one type at a time.

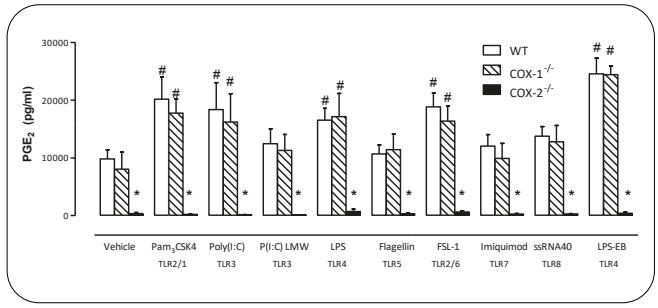

Figure 2: PGE2 expression in proliferating mouse fibroblast (wild type, COX-1-, COX-2-) following stimulation of different TLRs. Copyright : Republished with permission. From Wright, W. et al. (2013) Cyclooxygenase and cytokine regulation in lung fibroblasts activated with viral versus bacterial pathogen associated molecular patterns. Prostaglandins & Other Lipid Mediators. Volume 107, pages 4-12.

The results indicated PGE2 expression in mouse tissues was driven differently by COX-1 and COX-2 depending on the nature of those tissues. Fresh lung tissues depended on COX-1 only, while proliferating lung fibroblasts depended on COX-2 only (Figure 2) and lungs in culture relied on both.

Additional PGE2 assays in human lung fibroblasts showed stimulation of TLR3 with poly(I:C) resulted in the highest expression level of PGE2 (not shown). This was consistent with previous finding of TLR3 downregulation in idiopathic pulmonary fibrosis patients, and further suggest that the pathogenesis of that disease may involve a weakened TLR3 signaling.

TG2-Fibronectin interaction in ECM

Yakubov, B. et al. *PLoS One. 2014; 9(2): e89285*

Tissue transglutaminase TG2 is involved in cell motility and cell adhesion to extracellular matrixes via interaction with integrin and fibronectin (see Figure 1 for integrin and fibronectin roles in adhesion). Study of this interaction and identification of inhibitors could yield promising therapeutic innovation in the management of cancer and fibrotic disorders.

In a 2014 study, Yakubov et al. designed and built a protein-protein interaction assay using AlphaLISA reagents (Figure 3), with the purpose of using it as a screening platform for TG2-Fibronectin inhibitor identification.

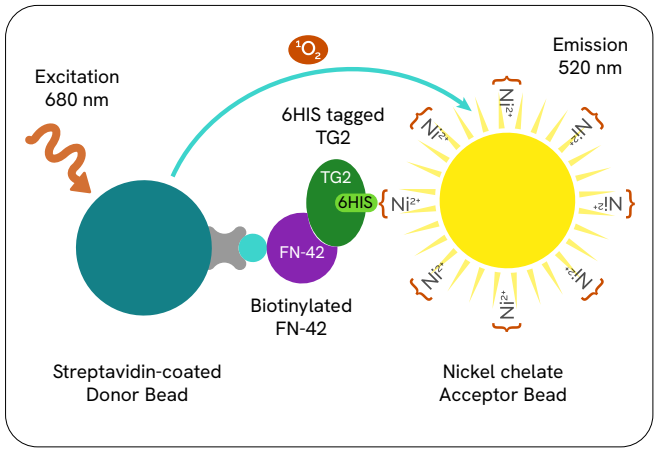

Figure 3: Assay format – The fibronectin segment that binds TG2 (FN-42) is biotinylated while TG2, is tagged with 6-His. AlphaLISA Donor and Acceptor beads are respectively coupled with streptavidin and nickel chelate respectively. The interaction of FN-42 and TG2 brings the Donor and Acceptor beads in proximity which allows energy transfer between the two and triggers the fluorescent signal. The addition of inhibitors. Take from: Yakubov, B. et al.

Using this assay, the authors screened 10000 compounds from the Chemical Genomics Core Facility at Indiana University (Figure 4) and confirmed 77 hits with ELISA.

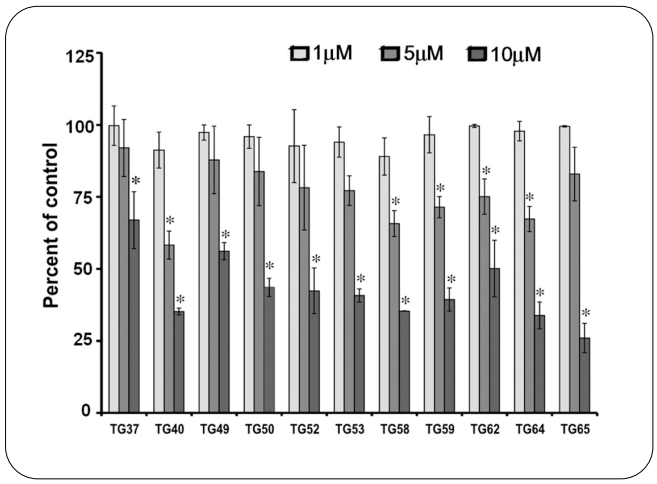

Figure 4: Dose dependent inhibition of TG2-FN interaction for several compounds Adapted from: Yakubov, B. et al

Those 77 were further investigated in cell culture-based assays targeting adhesion to fibronectin, migration and invasion capacity of ovarian cancer cells. One of those compounds, TG53, was found to exhibit potent inhibition of all and could be further developed as a potential inhibitor for ovarian cancer dissemination, the authors said.

Procollagen I to MMP-1 Ratio in malignant myocardial fibrosis

Eiros, R. et al. *J Clin Med. 2020 Feb; 9(2): 404.*

Hypertensive patients with heart failure are commonly prone to malignant myocardial fibrosis (mMF), which results in myocardial stiffness and diastolic dysfunction (DD).

This fibrotic disorder is characterized by a combination in serum of high pro-collagen type I (PICP) and a low ratio of carboxy-terminal telopeptide of collagen type-I to matrix metalloproteinase 1 (CITP:MMP1). This combination is very characteristic of an imbalance between extracellular matrix (ECM) constituents, which are high, and matrix metalloproteinase degrading the ECM, which are low.

Patients with chronic kidney diseases (CKD) are also prone to mMF and DD but the ties of CKD to those disorders have not been described in detail.

In this 2020 study, Eiros et al. aimed to improve our understanding of these ties and how CKD impacts the malignant myocardial fibrosis factor combination. They used a set of hypertensive patients split along two criteria

- Presence or absence of heart failure with preserved ejection fraction (HFpEF)
- Presence or absence of chronic kidney disease (CKD)

In that patient population, the authors measured the three markers of the mMF combination factors. PICP and CITP were assessed with Orion Diagnostica and Quidel Corporation solutions respectively while total MMP-1 was measured using Revvity's AlphaLISA corresponding kit.

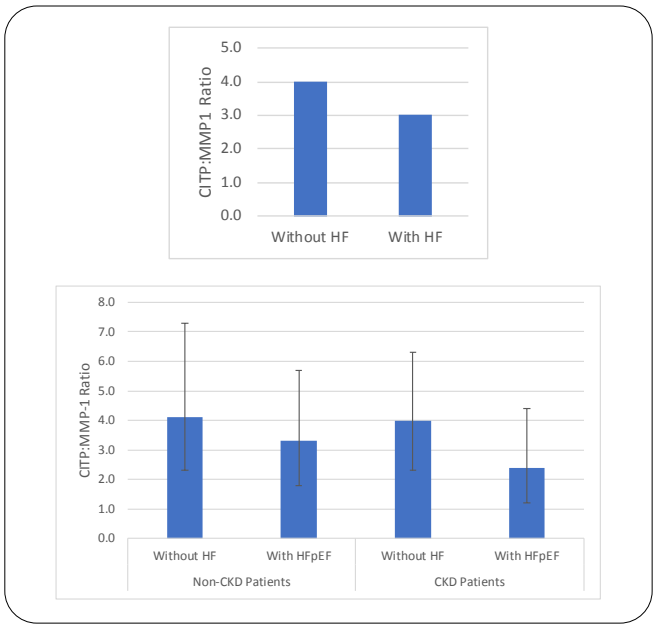

Figure 5: CITP:MMP-1 ratio in patients with or without chronic kidney disease and with or without heart failure. Adapted from: Eiros, R. et al. Top – p value <0.001. Bottom – non-CDK p=0.06, CDK p=0.004.

Patients with HFpEF were found to have lower CITP:MMP-1 ratios, which indicates higher occurrence of malignant myocardial fibrosis (mMF). Results also indicate that CKD further lower that ratio in HFpEF patients and those had the lowest ratio of all. This indicates that CKD may contribute to the development of mMF in HFpEF patient via the alteration of serum biomarkers levels.

Conclusion

The growing understanding of fibrotic disorders has led the latest research and therapies to investigate and address the delicate balance of inflammatory mediators, extracellular matrix constituents, adhesion mechanism and matrix degradation that rules wound healing and scarring processes.

HTRF and AlphaLISA assays have been successfully used to that end for years and demonstrated an acute relevance for the various requirements and formats of drug discovery.

The publications featured in this note report the effective implementation of such assays for investigating fibrosis development, as well as running experiments that improve the understanding of potential drug candidates.

References

1. Wright, W. et al. Cyclooxygenase and cytokine regulation in lung fibroblasts activated with viral versus bacterial pathogen associated molecular patterns. Prostaglandins & Other Lipid Mediators. Volume 107, pages 4-12 (2013)
2. Yakubov, B. et al. Small Molecule Inhibitors Target the Tissue Transglutaminase and Fibronectin Interaction. PLoS One. 2014; 9(2): e89285
3. Eiros, R. et al. Does Chronic Kidney Disease Facilitate Malignant Myocardial Fibrosis in Heart Failure with Preserved Ejection Fraction of Hypertensive Origin? J Clin Med. 2020 Feb; 9(2): 404.

Products used

- HTRF PGE2 detection kit
- AlphaLISA MMP-1 detection kit
- AlphaLISA toolbox reagents

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
www.revvity.com

For a complete listing of our global offices, visit www.revvity.com