# Regulation of Collagen-Rich Matrix Remodeling in Wound Healing, Inflammation and Cancer

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**Abstract:** CD44 plays multiple roles in the context of inflammation and cancer including leukocyte recruitment, activation, and intratumoral migration [1,2]. CD44 also plays a role in the uptake of its principal ligand, hyaluronic acid (HA), and accumulation of soluble factors involved in stromal cell activation [1,2]. The role of CD44 in matrix remodeling is less well understood. Wound healing, inflammation and the microenvironment of solid tumors are all characterized by the accumulation and remodeling of HA and collagen-rich extracellular matrices. We have found that genetic deletion of CD44 results in increased fibrillar collagen accumulation in each of these conditions and that this phenotype is recapitulated in isolated fibroblast-derived matrices generated by CD44-deficient fibroblasts compared to wild-type fibroblasts *in vitro*. The mechanisms underlying CD44-mediated matrix remodeling and the consequences of altered matrix remodeling in each of these conditions in CD44-deficient mice compared to wild-type mice were discussed.

## OVERVIEW

The overlap between many of the pathophysiologic processes involved in wound healing, chronic inflammatory and fibrotic diseases as well as cancer has been well characterized [3]. Similar analogies arguably apply in the context of autoimmunity. The shared hallmarks of all these pathologic conditions include a dysfunctional vasculature, angiogenesis, stromal cell activation and matrix remodeling. These processes are all highly regulated by cell-cell interactions involving epithelial cells, inflammatory and immune cells, endothelial cells, mesenchymal stromal cells such as fibroblasts and the hematogenous and lymphatic vasculatures [3]. These processes are also dependent on cell-matrix interactions. Matrix remodeling is a quintessential feature of all these processes that modulates biomechanical, biophysical and biochemical signaling that governs cell behavior to orchestrate the integrated response. The transmembrane adhesion receptor CD44 can mediate both cell-cell and cell-matrix interactions through its regulated affinity for HA and/or as a docking platform for matrix proteins, matrix modifying enzymes and growth factors [1,2] (Figure **1**). Myriad studies have implicated CD44 in linking extracellular cues to intracellular signals that impact cytoskeletal organization, proliferation, angiogenesis and cell polarity. Furthermore, post-translational modification, splicing, and proteolysis of membrane bound CD44 can alter CD44-HA signaling and CD44 activity. To define the impact of the many functions of CD44 on wound healing, inflammation/fibrosis and cancer, we compared responses and outcome in each of these contexts between wild-type and CD44-null mice. Results of these studies were presented.

## IMPACT OF CD44 ON CUTANEOUS WOUND HEALING

Cutaneous wound healing occurs through blood clotting, inflammation, re-epithelialization and tissue remodeling [4,5]. Stromal cell-dependent tissue remodeling involves dynamic restructuring of the fibrillar collagen rich ECM. Typically, the response to injury is self-limiting and leads to restoration of normal skin function and biomechanics. However, persistent accumulation of fibrillar collagen, the principal ECM protein found in the skin, can result in hypertrophic scar formation and highly compromised skin function and aberrant biomechanical properties compared to normal skin [6]. HA, the predominant glycosaminoglycan associated with the injury response, is upregulated early following injury and persists during wound healing [7,8]. Furthermore, evidence suggests that HA regulates collagen accumulation and impacts collagen architecture and thereby influences the outcome of wound healing. Thus, defining mechanisms that govern ECM dynamics is vital to understanding normal versus pathological hypertrophic scarring and developing approaches to prevent scarring.

Although CD44, the principal receptor for HA has been implicated in an array of inflammatory and fibrotic processes such as leukocyte recruitment, T-cell extravasation, and HA metabolism, the role of CD44 in cutaneous wound healing and fibrillar collagen accumulation remains unknown. To define the role of CD44 we compared wound healing in wild-type (WT)

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E-ISSN: 2310-9874/17 

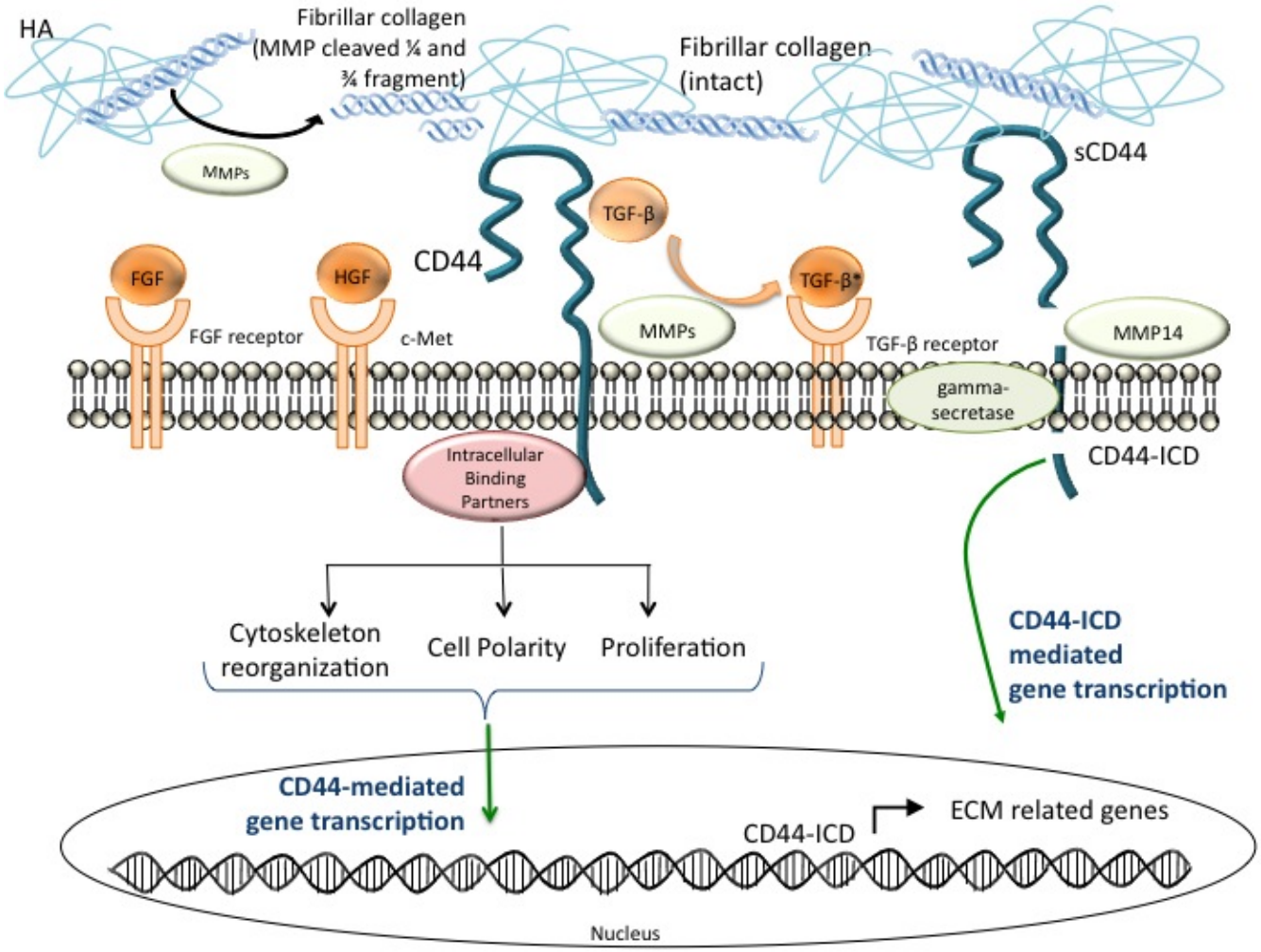


**Figure 1: Mechanisms of CD44-dependent cell-cell and cell matrix interactions.** CD44 is a cell surface adhesion receptor that plays a vital role in cell-ECM and cell-cell interactions through association with its primary ligand hyaluronic acid (HA), by functioning as a docking platform for matrix metalloproteinases (MMPs) and growth factors, by integration of a soluble form of its extracellular domain (sCD44) into the matrix, by signal transduction and/or by proteolytic release of its intracellular domain (CD44-ICD) that can regulate gene transcription. The extracellular domain of CD44 contains a Bx7B HA binding motif. CD44-HA binding of the transmembrane form of the receptor allows for the retention of HA on cell surface as major component of the glycocalyx and HA synthesis and turnover. The extracellular domain of CD44 is subject to extensive alternative splicing as well as multiple post-translation modifications including glycosylation and sulfation that can modulate its ligand affinity and activity. The intracellular domain of CD44 is itself subject to phosphorylation [11,12] and regulates phosphorylation of CD44 intracellular binding partners. The intracellular domain of CD44 can form complexes with merlin and ezrin/radixin/moeisin (ERM) [11,12]. Phosphorylation of ERM induces actin cytoskeleton remodeling and cell proliferation. Dephosphorylated merlin binds to CD44-ICD [13,14] and initiates cell cycle arrest by inhibiting Rac GTP, which is required for cyclin D1 induction. CD44 can also act as a docking platform for various proteins, enzymes, and growth factors that play a crucial role in ECM deposition and assembly such as fibroblast growth factor (FGF), collagen, osteopontin, and matrix metalloproteinases (MMPs) [2,15]. Collagenases, MMP9 and MMP7, have been shown to co-precipitate with CD44, even in the presence of CD44-HA blocking antibodies [16,17]. Additionally, optimal activation of TGF-β, a vital growth factor implicated in stromal cell activation and collagen deposition, is CD44-dependent [18]. In the context of a tumor microenvironment, hepatocyte growth factor (HGF)-induced cMet activation depends on CD44 [19]. The extracellular domain of CD44 (sCD44) released upon MT1-MMP-dependent cleavage can retain ligand affinity and can compete with membrane bound CD44 for HA binding, therefore altering CD44-HA dependent signaling and the extracellular milieu [20]. Under elevated conditions of dynamic matrix remodeling or high proteolytic activity, the release of sCD44 is followed by presenilin 1-dependent gamma secretase mediated proteolysis of the intracellular domain of CD44 thus liberating CD44-ICD [13]. CD44-ICD translocates to the nucleus and can induce transcription of various ECM related genes including CD44 itself, MMP9, MMP2, and Hif1α.

and CD44 global knockout mice using a punch-biopsy injury model. We found that CD44-deficient mice exhibit increased collagen synthesis leading to increased accumulation of fibrillar collagen at wound closure compared to WT mice. This increase in fibrillar collagen accumulation persisted after wound closure thus, recapitulating a more severe scarring phenotype. These data indicate that CD44 plays a previously

unknown role in fibrillar collagen accumulation during the response to cutaneous injury. We are currently investigating the mechanisms by which CD44-mediated signaling and/or CD44-ECM interactions regulates the dynamics of fibrillar collagen and thereby contributes to normal wound repair and the impact of CD44 deletion on the functionality of scar tissue.

## ROLE OF CD44 IN PANCREATITIS AND PANCREATIC CARCINOGENESIS

Pancreatitis is a well-known risk factor for the development of pancreatic cancer [9]. We used an experimental model of caerulein-induced acute pancreatitis to investigate the role of CD44 in the context of pancreatitis. We found that early pancreatitis was attenuated, and that resolution of pancreatitis was accelerated, in CD44-null compared to wild-type mice.

Excess accumulation of HA is a hallmark of pancreatic ductal adenocarcinoma (PDA) and recent evidence indicates that HA in this context confers resistance to therapy [10]. There are multiple potential approaches to target this increased HA accumulation including inhibition of HA synthesis, depletion of HA by treatment with hyaluronidases and blocking receptor-mediated HA signaling. To determine if targeting CD44 may provide a novel therapeutic approach to the treatment of PDA and to overcoming resistance of this disease to conventional therapies, we investigated the impact of genetic deletion of CD44 on tumor growth in an orthotopic transplant model of PDA. We found that CD44-null mice exhibited reduced tumor burden that was associated with an increase in collagen accumulation. Current studies are focused on the mechanisms by which CD44 exacerbates injury-induced pancreatitis and supports the growth of pancreatic cancer.

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Received on 01-11-2017 Accepted on 30-11-2017 Published on 31-12-2017

DOI: https://doi.org/10.12970/2310-9874.2017.05.07