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Review

Role of discoidin domain receptor 2 in wound healing

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Summary. Until recently, collagen interactions with cells had been ascribed to integrins. The identification of the Discoidin Domain Receptor (DDR) family as collagen receptors represents a new paradigm in the regulation of collagen-cell interactions. How DDR signaling is biochemically linked to specific cell regulatory functions remains largely unknown. Moreover, the characteristic slow and substained phosphorylation of DDRs and the elevated expression of DDR2 in the myofibroblasts of healing wounds suggest a role for DDR2 in physiological and pathological wound healing. In fact, DDR2 signaling regulates cell proliferation and extracellular matrix synthesis, which are key aspects of fibroblast contribution to tissue healing. In this review we summarize evidence in favor of this concept.

Key words: Collagen, Discoidin domain receptors, Myofibroblast, Receptor, Tyrosine kinase, Wound healing

Introduction

Injury to mammalian skin initiates a well-coordinated series of events that culminate in the reconstitution of the injured body site. The process of skin wound healing starts immediately after injury, when the fibril clot is formed by a provisional matrix for immune cells, fibroblasts and endothelial cells that are attracted to the wound site from the circulation or migrate into the clot from the wound edges (Müller et al., 2012). Fibroblasts are the major effectors of wound repair and regenerative healing because they orchestrate cellular recruitment to healing wound, collagenous extracellular matrix (ECM) deposition and the contraction of the resulting scar. Collagen receptors located in the plasma membrane of the fibroblasts mediate their interaction with the ECM, and their downstream signaling trigger in the whole healing process.

Until recently, the integrin family receptors were considered the main collagen receptors, but since the discovery of the implication of the receptor tyrosine kinase subfamily of Discoidin Domain Receptors (DDR1 and DDR2) in collagen signaling, a whole new field of research in wound healing has been opened to clarify the healing process. Here we will review the implication of DDRs in skin wound healing, with special emphasis on Discoidin Domain 2 (DDR2).

Steps in physiological skin wound healing

Skin wound healing is a dynamic interactive response that involves at least three overlapping tightly coordinated steps: An inflammatory reaction, a proliferative step allowing re-epithelialization and formation of granulation tissue and a maturation phase (Singer and Clark, 1999) (Table 1). The first invading immune cells are neutrophils, followed by monocytes/macrophages, mast cells and lymphocytes. These immune cells are an important source of various cytokines and growth factors, which are released upon injury or produced in response to signals present in the wounded tissue. Immune cells, in particular neutrophils and macrophages, also secrete proteolytic enzymes and reactive species as a defense against invading microorganisms, and they efficiently phagocytose cell debris and apoptotic cells (Martin, 1997; Gurtner et al., 2008; Muller et al., 2012).

After the initial inflammatory phase, the formation of new tissue (or proliferation phase) is initiated. As in the other phases of wound healing, steps in the proliferative phase do not occur in a series but rather partially overlap in time. The provisional new tissue is initially hyperplastic and poorly differentiated, but re-differentiation eventually occurs, which is accompanied

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by re-formation of a functional epidermal barrier. In parallel, dermal repair occurs, which involves migration and proliferation of fibroblasts at the wound edge. A large percentage of the wound fibroblasts transdifferentiate into myofibroblasts (Müller et al., 2012), characterized by their expression of features typical of smooth muscle cells, such as actin filament bundles (stress fibers) and an active, proliferative and synthetic status (Gabbiani et al., 1972). Then, endothelial cell sprouting occurs at the wound edge, resulting in the formation of a new vascular network. In parallel, lymphangiogenesis and re-innervation occur. The resulting provisional tissue is often called granulation tissue, because the multiple new capillaries provide a granular appearance of the newly formed stromal tissue. Granulation tissue comprises both cellular and collagenous extracellular matrix components. Contraction of the wound is initiated at this step.

The final phase of the wound healing process, or maturation phase, is characterized by apoptosis of excessive number of fibroblasts and endothelial cells. Remodeling of the extracellular matrix persists in this phase (Gurtner et al., 2008; Müller et al., 2012). In fact, the onset of the maturation phase is defined by an equal production and degradation of the collagenous matrix, so there is no net collagen gain. Also, type III collagen, which is prevalent during proliferation, is replaced by type I collagen. Moreover, at this step collagen is cross-linked and organized to gain tension strength. The onset of the maturation phase may vary extensively, and can last for a year or longer, depending on the wound type.

Table 1. Steps in skin wound healing.

Step	Main Cell Type	Functional Mechanism
Inflammation	Macrophages	Phagocitosis Chemotaxis ECM degradation
Proliferation	Epithelial cells	Re-epithelization ECM degradation
	Endothelial cells	Angiogenesis
	Fibroblasts	Collagen synthesis >> Collagen degradation Contraction Collagen crosslinking
Maduration	Fibroblasts	Collagen aligment Collagen synthesis = Collagen degradation Apoptosis

Skin wound healing involves at least three overlapping tightly coordinated steps. A first step is characterized by an inflammatory reaction, where the macrophages are the main cell type. A second phase comprises a cell proliferation where re-epithelialization, fibroblast recruitment and synthesis of granulation extracellular matrix and angiogenesis takes place. In the maturation step apoptosis of excesive cells and reorganization of the extracellular matrix improves tensile strenght of the healed skin.

Skin fibroblasts are the main cellular type responsible for wound ECM synthesis and remodeling

Collagens are key extracellular matrix components of the granulation tissue because they bring support for cell ingrowth and differentiate and serve as a scaffold for cell migration (McClain et al., 1996). Collagens are mainly synthesized and laid down by the wound myofibroblasts. They synthesize fibrillar collagens (i.e. types I and III), which are the main ECM component of the healing tissue, and non-fibrillar collagens (i.e. types IV and VI) which conform the basement membrane that play critical roles in epithelial cell polarity and function (Boudreau and Bissel, 1998; Lelievre et al., 1998). While their exact origin remains unknown, it is well accepted that the main source of wound myofibroblasts are fibroblasts from adjacent uninjured tissue close to the wound edge (Gabbiani et al., 1972) and to a lesser extent, bone marrow-derived circulating adult stem cells/precursors (Iwaisako et al., 2012). By the end of the first week after injury, myofibroblasts are the main cells in the wound.

The collagenous matrix undergoes continuous remodeling along the healing process. Early wounds are mainly composed of collagen type III. As the healing process develops and increased strength of the wound is required, collagen type III is substituted by collagen type I. This process is achieved by a shift in the myofibroblast synthesis program and by active degradation of the existing collagenous extracellular matrix by specific collagenases. Wound myofibroblasts synthesize matrix metalloproteinase 2 (MMP-2, gelatinase A), the predominant protease responsible for wound remodeling (Okada et al., 1997). MMP-2 is also likely to drive fibroblasts to a more synthetic phenotype. As a result, a microenvironment favoring continued fibrillar collagen production is created (Martin, 1997; Olaso et al., 2002).

Once the collagen production ceases, wound myofibroblasts secrete specific cross-linking enzymes to rearrange the originally disorganized collagen fibers and align them along tension lines (Duffield et al., 2013).

Epithelial cells and macrophages also contribute to ECM remodeling; they synthezise basement membrane-degrading proteases (e.g. MMP-9), which are important during inflammation and early re-epithelialization. Depletion of macrophages during the re-epithelialization phase significantly disturbed the transition from the proliferative stage to the maturation stage of repair response (Lucas et al., 2010), suggesting a role for macrophages in the resolution of the inflammatory phase (Delavary et al., 2011).

Collagen receptors in skin wound healing

Collagens are the most abundant ECM constituents in mature, physiological tissues and are important in dynamic processes, such as tissue reorganization,

morphogenesis, immunomodulation, hemostasis and thrombosis and wound healing. Even though collagens are found in all metazoans and are considered to have contributed to the early evolution of multicellular animals (Exposito et al., 2010), the structurally diverse group of transmembrane collagen receptors appear much later, integrin and DDR families being the most widely expressed collagen receptors in vertebrates (Valiathan et al., 2012). Integrins are essential mediators of activation in response to injury, infection and inflammation (Parks, 2007). Once the ligand binds the integrin, the signal information is transduced via outside-in signaling to regulate various responsive processes, such as proliferation, gene expression, and cell survival. Integrins are a large family of heterodimeric surface proteins composed of an α-chain and β-chain, and different combinations of these subunits form different transmembrane receptors, which in turn bind a wide variety of compounds in the extracellular space: matrix proteins, circulating factors, latent growth factors, proteinases, other cell surface molecules, virus particles, and more (Wehrle-Haller and Imhof, 2003). Humans have 24 combinations of αβ subunits (Johnson et al., 2009) only four of them being collagen-binding integrins; α1β1, α2β1, α10β1 and α11β1 (Popova et al., 2007; Barczyk et al., 2010; Eitinger, 2011). In the skin, integrin signaling modulates cell proliferation and migration (Gamble et al., 1993; Larjava et al., 1993; Martin, 1997; Baum and Arpey, 2005). They also function as mechanotransducers of the tensile strength exerted by the healing matrix (Ingber, 2006; Wells, 2008) to further activate downstream signaling that drives fibroblast contraction of the wound (Wang et al., 1998; Kagan and Li, 2003; Payne et al., 2007).

Despite the important role of integrins, several aspects of cell responses to collagen are not altered in integrin-deficient fibroblasts. For example, the blockage of α2β1 integrin with a specific antibody does not inhibit completely MMP-2 activity, indicating that another signaling pathway is participating in the ECM remodeling (Théret et al., 1999). In this regard, Olaso et al observed that the non-integrin discoidin domain receptor 2 (DDR2) modulates MMP-2 expression in fibroblasts (Olaso et al., 2002) (see below). Furthermore, Discoidin Domain Receptor 1 (DDR1) is tyrosine phosphorylated and activated by cell binding to collagen, even in the presence of β1 integrin blocking antibodies (Vogel et al., 2000).

Receptor tyrosine kinases in skin wound healing

Soluble growth factors are key regulators of wound repair because they control cell migration, proliferation, differentiation and survival at different stages of the healing process (Müller et al., 2012). Growth factors are synthesized by proliferating cells of the granulation tissue, creating a positive loop for cell growth. As myofibroblasts become the larger population in the granulation tissue, their role in the synthesis and release of growth factors increases. In a broad sense, growth factors encourage fibroblast proliferation, migration to the wound bed, and production of ECM molecules. Hypoxia also contributes to fibroblast proliferation and excretion of growth factors, though too little oxygen will inhibit their growth and deposition of ECM components, and can lead to excessive, fibrotic scarring.

The majority of soluble growth factors involved in tissue healing, such as PDGFβ, TGFβ and VEGF, exert their cell response by a direct interaction with cell surface receptors of the receptor tyrosine kinases (RTK) family. RTKs have emerged as key regulators of critical cellular processes, such as proliferation and differentiation, cell survival and metabolism, cell migration, and cell-cycle control (Lemmon and Schlessinger, 2010). Humans have 58 known RTKs, which fall into 20 subfamilies. All RTKs have a similar molecular architecture, with ligand-binding domains in the extracellular region, a single transmembrane helix, and a cytoplasmic region that contains the protein tyrosine kinase (TK) domain plus additional carboxy (C-) terminal and juxtamembrane regulatory regions. Interestingly, the mechanism of RTK activation and intracellular signaling pathways that they trigger are highly conserved in evolution, which is consistent with the key regulatory roles that they play. Furthermore, numerous diseases result from genetic changes or abnormalities that alter the activity, abundance, cellular distribution, or regulation of RTKs. Moreover, mutations in RTKs and aberrant activation of their intracellular signaling pathways have been causally linked to cancers, diabetes, inflammation, severe bone disorders, arteriosclerosis and angiogenesis (Lemmon and Schlessinger, 2010).

In general, binding of a growth factor activates the RTK by inducing receptor dimerization (Ullrich and Schlessinger, 1990). RTKs contain a catalytic domain that autophosphorylates one or more tyrosine residues typically located in the non-catalytic region of the receptor. These phosphorylations lead to generation of docking sites for SH2 and PTB domains of signaling molecules that associate with the receptors (Vogel, 1999). For example, the RTK PDGFβ is present at the fibroblast conforming the wound site during the early phase of healing and it is related with signaling molecules such as phospholipase C-γ, Src, Shc, and phosphatidylinositol 3-kinase. Moreover, reduced expression of PDGF and PDGFβ leads to impaired wound healing (Werner and Grose, 2003), suggesting that normal expression of these genes is required for an efficient wound healing process (Müller et al., 2012).

A new paradigm in cell-collagen interactions: the discoidin domain receptors are a subfamily of RTKS for collagen

During the search for new RTKs by homology cloning, a novel subfamily called Discoidin Domain Receptor (DDR) was discovered (Di Marco et al., 1993;

Johnson et al., 1993; Zerlin et al., 1993; Laval et al., 1994; Perez et al., 1994; Sanchez et al., 1994; Alves et al., 1995). The DDR subfamily is composed by two members: DDR1 and DDR2. Both DDRs are widely expressed in human and mouse tissues, but their distribution is mutually exclusive (Labrador et al., 2001). The principal differences between DDR1 and DDR2 are their tissue specificity and relative affinities for collagens. BiaCore analysis indicates that whereas DDR1 is activated by type I, IV, and V collagens, DDR2 is activated by type I collagen and to a lesser extent by type II, III, and V collagens (data kindly provided by C. Radziejewski, Regeneron Pharmaceuticals Inc. New York). DDR1 is expressed primarily in epithelial tissues and has been implicated in the growth of epithelial neoplasms, including breast cancer (Johnson et al., 1993). In contrast, DDR2 was initially observed in mesenchymal cells (Alves et al., 1995) and in stromal cells surrounding highly invasive DDR1-positive tumor cells (Barker et al., 1995). Interestingly, DDR2 expression is upregulated in dermal burns (Feezor et al., 2004), and in pathological wound healing such as fibrotic diseases of the lung (Avivi-Green et al., 2006), kidney (Rubel et al., 2014) and liver (Olaso et al., 2001).

The DDR subfamily differs from other members of the large RTK group in three key features:

1) Regarding their structure, they contain a unique homology domain to discoidin, a lectin expressed by the slime mold *Dyctiostelium discoideum* (Lai and Lemke, 1994). DDRs represent two distinct genes based on homology of the N-terminal domain, the DDR1 protein, which have been previously named DDR, TrkE, CAK, RTK-6 and MCK-10 and it is composed of five membrane-anchored isoforms generated by alternative splicing, and the DDR2, also named CCK-2, TKT, and Tyro 10 that is represented by a single isoform (Valiathan et al., 2012) (Fig. 1). All members of the DDR family share a 160-amino-acid-long amino-terminal discoidin homology domain followed by a single transmembrane region, a juxtamembrane region, and the catalytic tyrosine kinase domain. The DDR collagen-binding site is in the form of a trench created by five protruding loops at the "top" of the discoidin 1 domain, opposite the disulfide-linked chain termini. The identification of the GVMGFO motif as a DDR ligand enabled the crystal structure determination of a complex between the DDR discoidin 1 domain and a triple-helical peptide encompassing the GVMGFO motif (Carafoli et al., 2009). This structure revealed that the apolar GVMGFO motif, present in the fibrilar collagen I-III is accommodated in an amphiphilic binding pocket, and that several residues at the periphery of the GVMGFO peptide-binding interface are responsible for the distinctive collagen binding specificity of the DDRs (Valiathan et al., 2012) (see below).

2) Functionally, DDRs are the only RTks that signal in response to collagen. The identification of the DDR family as collagen receptors represents a new insight into the regulation of collagen-cell interactions in wound healing (Shrivastava et al., 1997).

3) The kinetics of DDR receptor activation by collagens differs significantly from other RTKs in response to their cognate ligands. For example, the tyrosine phosphorylation of DDR receptors is detected only after prolonged exposure to collagen (approximately 30 min), and then phosphorylation is sustained for an extended period (more than 16 h) (Vogel et al., 1997; Olaso et al., 2001) while standard RTK phosphorylation and dephosphorylation occurs within minutes. This unique slow-on, slow-off phenomenon and receptor specificity raise important questions about the nature of downstream intracellular signals mediating the effects of DDRs. While the molecular mechanism of collagen binding to the DDR's discoidin domain has been recently discovered (Carafoli et al., 2009), the activation of the cytosolic kinase domain is not clear. DDR1 activation by collagen results in binding of Shc1 to Tyr513, SHP-2 to Tyr703, Tyr796 and Tyr740, and the p85 subunit of PI3K to Tyr881 (Koo et al., 2006; Wang et al., 2006). Collagen binding also stimulates DDR1 interaction with Src (Lu et al., 2011). This discovery has important consequences because Src is the main mediator in cell-cell and cell-ECM adhesion (Hynes, 1992; Takeichi, 1995; Thomas and Brugge, 1997; Aplin et al., 1998) and in RTK signaling (Parsons and Parsons, 1997). Additional binding proteins such as Ras-GAP, SHIP-1 and STATs have also been identified (Vogel et

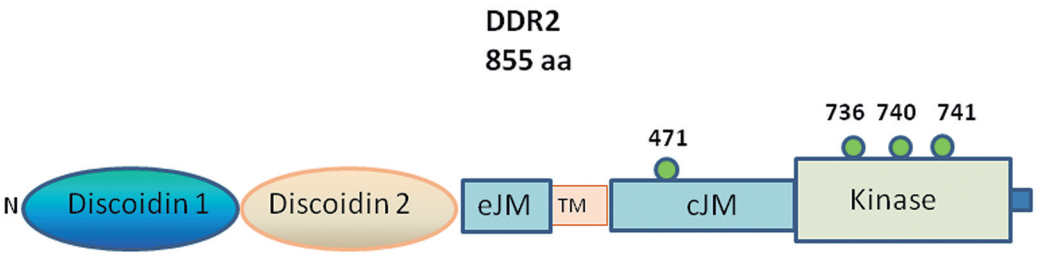

Fig. 1. Structure of the Discoidin domain 2 receptor structure. The ectodomain of DDRs contains a N-terminal discoidin domains, which included a discoidin 1 and an a globular domain discoidin 2. This discoidin 2 domain is followed by an extracellular juxtamembrane (eJM) region, a single transmembrane (TM) domain and an unusually large cytosolic juxtamembrane domain (cJM). A catalytic kinase domain follows the cytosolic JM domain followed by a short C-terminal tail. The mMain phosphorilation sites are shown (green circles).

al., 1997; Yang et al., 2005; Wang et al, 2006; Lemeer et al., 2012).

DDR2 signaling following collagen activation is poorly characterized. DDR2 activation requires the phosphorylation by Src of tyrosines Y736, Y740 and Y741 in the activation loop of the DDR2 tyrosine kinase domain (Ikeda et al., 2002; Yang et al., 2005). Substitution of Y740 for phenylalanine resulted in constitutive collagen independent DDR2 kinase activity suggesting that this residue plays a role in blocking DDR2 autophosphorylation (Yang et al., 2005). Following Src phosphorilation, intramolecular autophosphorylation of DDR2 at other tyrosine residues is poorly described. Ikeda and colleagues discovered that, as reported for DDR1 (Vogel et al., 1997) Shc associate with DDR2 (Ikeda et al., 2002), but the interacting region of Shc for DDR1 and DDR2 is different. Tyr-471 phosphorylation in the intracellular domain is critical for DDR2/Shc interaction, and shc phosphorylation at tyrosine residues Tyr- 239, Tyr-240, and Tyr-317 in its CH1 domain may also be necessary for the association with DDR2, particularly because these residues are preserved in all three isoforms of Shc (Pelicci et al., 1992). Interestingly, Tyr-239 and Tyr-240 residues in the CH1 domain of Shc are also phosphorylated by Src (Van der Geer et al., 1996). Moreover, activated DDR2 association with Shc results in increased MMP-2 promoter activity (Ikeda et al., 2002). A novel approach using phosphoproteomic analysis of DDR2-expressing cells has recently identified several candidate downstream signaling proteins, including SHIP-2 and PEAK1 (Iwai et al., 2013). Finally, DDR2 is involved in the regulation of FAK levels in vascular smooth muscle cells (Bhadriraju et al., 2009), and human lung fibroblast transmigration through collagen I-coated inserts is mediated by DDR2-associated signaling kinases JAK2 and ERK1/2, but not DDR1 (Ruiz and Jarai, 2010).

Defining the signal transduction pathways coupled to DDR activation downstream Sch/Src has become a challenging task. First, DDRs bind to multiple collagen types, which exhibit unique and common structural and biological properties, and consequently different collagen types may produce different receptor responses (Ongusaha et al., 2003). Second, collagens have multiple cell interaction sites that recognize different binding proteins (e.g. integrins) (Sweeney et al., 2008). Third, DDR signaling is cell/tissue type-specific and context-dependent (Ongusaha et al., 2003; Wang et al., 2006, 2009; Lu et al., 2011; Suh and Han, 2011). And fourth, DDRs may act with other signaling receptors, which include the Wnt5a/Frizzled (Jönsson and Andersson, 2001; Dejmek et al., 2003; Roarty and Serra, 2007), Notch1 receptors (Kim et al., 2011) and the insulin receptor (Huang, 2012; Iwai et al., 2013). As a whole, the collected data indicates that DDRs are part of the signaling networks that translate information from the extracellular matrix acting as key regulators of cell-matrix interactions. Under physiological conditions, DDRs control cell and tissue homeostasis by acting as collagen sensors, transducing signals that regulate cell polarity, tissue morphogenesis, and cell differentiation (Valiathan et al., 2012).

Interaction between integrins and DDRS

Because integrins and DDRs share common ligands and downstream signaling intermediates, an interaction between both collagen receptors was previously suggested, but the functional nature of these interactions has not been defined. It seems possible that integrins and DDR2 are co-localized and aggregated as they hold collagen as a common ligand, which may enable their signaling pathways to converge downstream. Two findings support this notion: First, the Src pathway, already known to be involved downstream of integrin signaling, is requisite for maximal DDR2 tyrosine phosphorylation and for engagement of the Shc adaptor protein (Ikeda et al., 2002). Second, Staudinger and colleages demonstrated in 2013 that DDR1 over-expression is associated with increased glycosylation of the β1 integrin subunit, which reduced collagen binding when blocked by deoxymannojirimycin (Staudinger et al., 2013). This result may indicate that DDR1 regulates β1 integrin interactions with fibrillar collagen. On the other hand, previous studies suggested the opposite. For example, blockage of β1 integrin does not inhibit DDR1 phosphorylation following cell binding to collagen (Vogel et al., 2000).

DDR2 deficient mice show delated cell growth

To establish the physiological role of DDR2, Labrador and colleagues generated DDR2-deficient mice (DDR2-/-) (Labrador et al., 2001). The Ddr2 gene was disrupted by replacing a neomycin resistant cassette instead of the exon K1, which coded the ATP binding region of the kinase domain in embryonic stem cells (ESC). Then, several targeted ESC clones were microinjected into C57BL/6J blastocysts to generate chimeras, and chimeric males were mated to 129/Sv females. The obtained offspring were screened for germline transmission, finally obtaining a germline-targeted mice from only one ESC clone.

DDR2-/- mice developed a progressive skeletal phenotype characterized by shortening of long bones, irregular growth of flat bones and a shorter snout. DDR2 expression shows a characteristic pattern of expression corresponding to the tibial chondrocyte columns in the proliferative region of the growth plate. Bone growth depends mainly on chondrocyte proliferation and differentiation. While development of the different lineages appeared normal in DDR2-/- mice, proliferating chondrocytes in the growth plates of DDR2-/- mice, where DDR2 should be expressed, showed a reduction in the proliferation index, which accounts for the small size of bones.

Interestingly, the slie mice, which carry a

spontaneous, autosomal-recessive deletion mutation spanning Ddr2 exon 1-17 are also dwarfed (Kano et al., 2008).

To define the sites of its *in vivo* action, Labrador and colleagues analyzed DDR2 protein expression and activity in mouse tissues (Table 2). DDR2 protein was found in most tissues, and the highest levels of phosphorylation were found in lung, ovary and skin. Interestingly, DDR2 expression did not necessarily correlate with its phosphorylation levels, and given the high level of DDR2 phosphorylation in the skin, the role of the receptor in this tissue was further analyzed (see below).

DDR2 modulate s skin fibroblast proliferation, migration, and collagen type I and MMP-2 synthesis

In vitro experiments using DDR2-/- skin fibroblasts demonstrated that absence of DDR2 causes a significant reduction in the proliferation rate of DDR2-/- fibroblasts compared with wild-type. Furthermore, the diminished activities of DDR2-/- cells was restored to levels similar to those of wild-type cells by stably reconstituting DDR2, indicating that the anti-proliferaitve effect was due to lack of DDR2 expression (Olaso et al., 2001). Cultured DDR2-/- fibroblasts also exhibited a significant decrease in collagen type I production. Thus, a model was proposed where decreased expression of DDR2 reduced collagen production, which in turn diminished the amount of collagen accessible to DDR2, leading to reduced cell proliferation.

DDR2-/- fibroblasts also showed less in vitro migration ability through a basement membrane-like matrix compared with DDR2+/- cells. This decreased migration was due to reduced expression of MMP-2, as DDR2-/- fibroblasts completely lacked the capacity to transcriptionally activate the MMP-2 promoter. MMP-2 activity is required to degrade a basement membrane matrix *in vivo*, and loss of MMP-2 is probably the primary reason for reduced migration of DDR2-/- cells. It is uncertain whether loss of MMP-2 solely accounts for this phenotype, because type I collagen promotes migration of smooth muscle cells (Rocnik et al., 1998). It is also possible that other MMPs also essential for cell migration are altered in skin fibroblasts in the absence of

Table 2. Distribution of DDR2 in broadmouse tissues.

	Immunoprecipitation/Western Blotting	
	Anti-DDR2	Anti-PY
Brain	+	
Cerebellum		
Heart	++	
Kidney		
Liver	+	+
Lung	+++	++
Skeletal muscle	++	+
Ovary	+++	++
Skin	+++	++
Spleen	++	
Stomach	+++	+
Thymus	++	
Intestine	++	+

Labrador et al. (2001) analysed the DDR2 distribution in broad tissue by inmunopreciitation and western bloting with anti-DDR2 and anti-phosphotyrosine (Anti-PY) specific antibodies. Anti-PY reveals high levels of phosphorilated DDR2 protein in the skin DDR2 in mouse tissues was analyzed by inmunoprecipitation /Western Blotting using anti-DDR2 and anti-phosphotyrosine (Anti-PY) specific antibodies. Anti-PY reveals high levels of phosphorylated DDR2 protein in the skin.

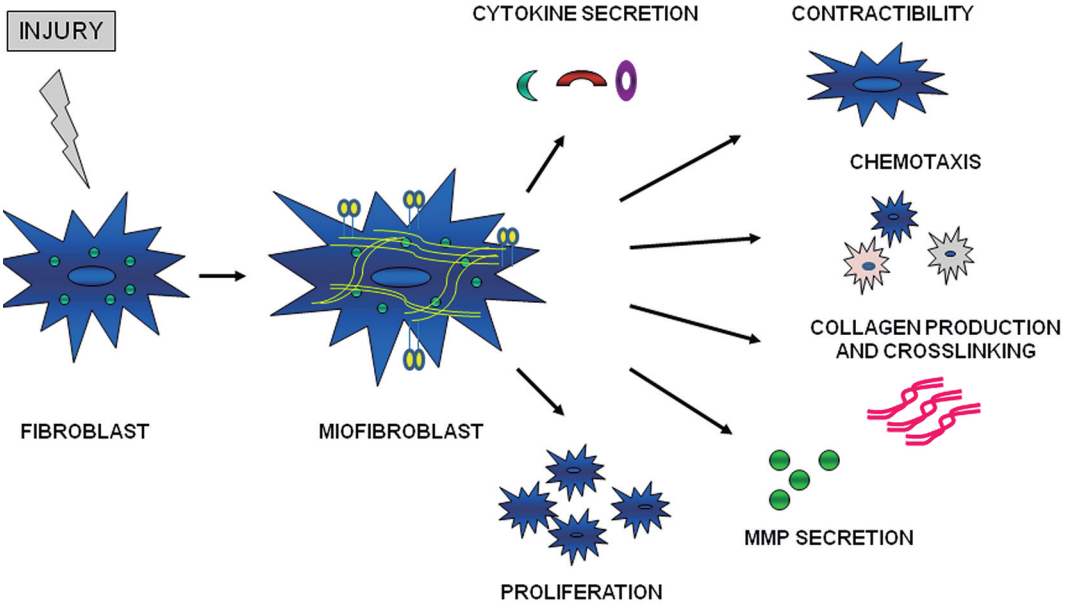

Fig. 2. DDR2 implication in fibroblast tTransdifferentiation in skin wound healing of Fibroblast. Resting dermal fibroblasts transdifferentiate into myofibroblastic cells in response to external stimuli such as tissue injury. Such myofibroblasts are characterized by de novo expression of actin filament boundless (stress fibers) and the collagen RTK DDR2. DDR2 signaling mediates the main aspects of myofibroblast involvement in wound healing such as synthesis of collagenous ECM, release of self-proliferation and chemotactic factors, collagen-degrading MMPs cross-linking enzymes and fibroblast contraction.

DDR2. For example up-regulation and activation of DDR2 by native type II collagen specifically induces the expression of MMP-13 by chondrocytes (Xu et al., 2005). Moreover, up-regulation of DDR2 is associated with overexpression of MMP-1 in synovial fibroblasts from rheumatoid arthritis patients (Wang et al., 2002).

In general, these studies established a requirement for DDR2 signaling in the proliferative phase of skin wound healing and suggest the important role of DDR2 in physiological and pathological scenarios where the ECM provides a signal for increased proliferation (Fig. 2). To further analyze DDR2 implication in tissue wounding, a model for in vivo skin wound healing was set and assayed (see below).

Abnormal experimental wound healing in DDR2-deficient mice: altered synthesis of ECM and ECM-related molecules

Wild type and DDR2-/- mice were submitted to experimental skin wounding (Olaso et al., 2011). Skin wounds healed more slowly in DDR2-/- mice than in wild type ones. As expected from the in vitro data (see above), DDR2-/- wounds showed reduced numbers of recruited fibroblasts, lower MMP-2 tissue levels and diminished collagen type I amounts. As discussed previously, fibroblast proliferation to the wound and remodeling of the ECM are key aspects of the formation of granulation tissue in physiological wound healing.

Fibroblasts also contribute importantly to the maturation step of a healing wound, where the contracting wound remodels the provisional ECM to one rich in highly cross-linked collagen type I to provide tensile strength, and realigns it along tension lines. Alteration in this phase may lead to formation of non-healing chronic wounds. Because DDR2-/- skin wounds showed impaired wound closure, an in vitro model for fibroblast contraction analysis was set. As expected, DDR2-/- skin fibroblasts activated by keratinocyte supernatants contracted collagen gels 40% less that their wild type ones. Mechanistically, the reduced expression of MMP-2 in the fibroblast lacking DDR2 may also explain the delayed closure of DDR2-/- defective wounds, because MMP-2 modulates *in vivo* and *in vitro* fibroblast contraction (Bullard et al., 1999; Beare et al., 2003; Mirastschijskia et al., 2004).

Finally, DDR2-/- skin wounds showed impaired recovery of tensile strength, which may be ascribed to a defective ability of DDR2-/- fibroblast to synthesize the collagenous ECM in the newly formed healing tissue. Reduction in the collagen content of the DDR2-/- skin wounds correlates with an altered expression of MMPs, diminished amounts of cross-linking enzymes lysyl oxidase (LOX) and lysyl hydroxylase 1 (LH1), and deficient expression of SPARC (also known as osteonectin). Reduced levels of LOX may be a hallmark of DDR2-deficient fibroblasts under wounding conditions, because miofibroblasts of fibrotic DDR2-deficient livers also express reduced LOX mRNA levels (Olaso, unpublished data).

Despite the important effects of collagen interactions on fibroblast function, the target genes downstream of activated DDRs and their physiological significance are largely unknown. Using a novel method to dissect signaling pathways induced by ECM receptors, Faraci and colleagues (Faraci et al., 2003) analyzed gene expression in human fibrosarcoma HT1080 and mouse fibroblast NIH3T3 cell lines over-expressing DDR1 or DDR2. To do so, they used microarrays specific for human and mouse genes coding for ECM proteins or ECM-interacting factors, including 29 collagens, 20 integrins and 17 MMPs. Ten percent of the genes studied were up- or down-regulated more than twofold in response to signals generated by over-expressing DDRs. Based on Faraci's results, Olaso and colleagues utilized comparative expression analysis of ECM-related genes in skin fibroblasts derived from wild type or DDR2-deficient mice. As expected from data obtained from protein expression analysis, Col I (α1) and MMP-10 gene expression were reduced in DDR2-deficient fibroblasts. On the other hand, several genes upregulated in HT1080 and/ or NIH3T3 DDR2-overexpressiong cells such as col VIII and Fibrillin were also down-regulated in DDR2-deficient cells, and genes down-regulated by DDR2 overexpression were upregulated in DDR2 deficient fibroblasts. Expression of α2 integrin and MMP-2 mRNAs did not correlate in both models, indicating that removal of Ddr2 gene does not elicit necessarily the opposite cell responses than stable overexpression of DDR2 protein.

In conclusion, altered DDR2 signaling in skin fibroblasts affects skin wound healing and tensile strength through various interconected mechanisms: (1) chemotactic migration to the wounded area and proliferation, (2) synthesis and remodeling of the wound matrix, (3) three-dimensional organization of the wound matrix by collagen cross-linking and (4) fibroblast contraction of the healing wound.

Future directions

1. From the data accumulated so far, it is tempting to envision DDR2 as a therapeutical target aimed at accelerated wound healing and reduced keloid formation. One may speculate on the use of specific small molecule compounds, which either interfere with the binding of collagen to DDR or which act as specific inhibitors of DDR tyrosine kinase activity (Day et al., 2008). To this regard, a much deeper understanding of the mechanisms of collagen-receptor signaling is mandatory.

2. Impressive recent data on stem cell *plasticity*, or their ability to *de-differentiate* back into hematopoietic stem cells and/or *transdifferentiate* into non-lineage cells such as fibroblasts, point them out as an alternative therapeutic tool in the treatment of defective skin wound healing (Wong et al., 2013). Interestingly, hematopoyetic derived fibroblasts express DDR2 (Ebihara et al., 2006;

Abangan et al., 2010).

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