

Discoidin domain receptors: Microenvironment sensors that promote cellular migration and invasion

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ABSTRACT

Extracellular matrix (ECM) provides cells scaffolding for cell migration and microenvironment for various cellular functions. Collagens are major ECM components in tissue and discoidin domain receptors (DDR) are receptor tyrosine kinases (RTK) that recognise fibrillar collagens. Unlike other RTK, their ligands are solid ECM that are abundantly present in the pericellular environment in various tissue, and thus its activation and regulations are unique amongst RTK family. It is emerging that DDRs may be the sensors that monitor and detects changes in ECM microenvironment and determines the cellular fates upon tissue injuries. In this mini-review, recent findings on the role of DDRs as microenvironment sensor and their roles in cell migration and invasion are discussed.

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1. Introduction

In multicellular organisms, extracellular matrix (ECM) plays an essential role in maintaining functional tissue structures, as a scaffolding to support cell migration and as a reservoir for growth factors. Also, components of ECM can directly transmit signals, promoting cell survival and differentiation. Among various ECM proteins, collagen is the most abundant ECM molecules in our body and a major component of cellular microenvironment [1]. There are several classes of collagen binding receptors in mammals including Integrins, discoidin domain receptors (DDRs), glycoprotein IV, Leukocyte-associated Ig-like receptor-1, Mannose receptor family proteins including uPARAP (urokinase plasminogen activator receptor-associated protein)/Endo180 [2]. Among these molecules, there are two classes of receptors that are known to transmit collagen signals to the cells: collagen binding integrins [3] and discoidin domain receptors [4]. Integrins are heterodimers of non-covalently associated α and β subunits that is a major family of ECM receptors for cell adhesion [3]. Of the 24 distinct integrins in humans, four members including $\alpha1\beta1$, $\alpha2\beta1$, $\alpha10\beta1$ and $\alpha11\beta1$ are known to be collagen binding integrins [2]. They all have common $\beta1$ subunits and their ability to bind to collagen can be altered by their active- or inactive-conformation of the integrins that can be regulated by both outside-in and inside-out

signals [3]. This allows cells to be able to control cell adhesion in a spatiotemporal manner. On the other hand, the ability of DDRs to bind to collagen is spontaneous and is not influenced by signals from outside or inside. Therefore, it requires additional steps to control binding of DDRs to collagen as well as its signaling. In this mini-review, recent findings of their unique regulatory mechanism and involvement in cell migration and microenvironment recognition are discussed.

2. Structure of ddrs and their expression

DDRs are type I transmembrane proteins. Their domain structure consists of a collagen binding discoidin (DS) domain, a DS-like domain, an extracellular juxtamembrane (JM) region, a transmembrane region, an intracellular JM region and a tyrosine kinase (TK) domain. N-terminal DS domain is the collagen binding domain in both DDR1 and 2. DDR1 has five different isoforms due to alternative splicing: DDR1a-e. Among these isoforms, DDR1d and DDR1e are kinase-deficient due to a frame shift and truncation. DDR1a (876 amino acids) and DDR1b (913 amino acids) are commonly expressed DDR1 isoforms, and DDR1b has an insertion of 37 amino acids at the intracellular JM region of DDR1a sequence. DDR1c has additional 6 amino acids insertion in TK domain in DDR1b sequence. DDR2 does not have

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such isoforms and consists of 588 amino acids [4, 5]. DDR1 is generally expressed in epithelial cells and immune cells including mononuclear cells, activated T-cells. DDR2 is generally expressed in mesenchymal cells including fibroblasts and chondrocytes, but it was also shown to be expressed in neutrophils [4]. In normal cells, there are no examples that both DDR1 and DDR2 are expressed, but several de-differentiated epithelial cancer cells and fibrosarcoma cells are shown to express both DDR1 and DDR2 while well-differentiated epithelial cancer cells seem to express the only DDR1, but not DDR2.

3. Ligand specificity

DDRs are unique among RTK family in being bound and activated by solid ECM components, collagens. Monomer collagens consist of three polypeptide α chains forming right-handed triple helical structure. Some collagens are composed of three identical α chains (homo-trimeric collagens), while others have two to three distinct α chains (heteromeric collagens) [1]. These monomeric collagens further assemble fibers or sheet-like networks. The most abundant collagens in our body are fibrillar collagens (type I, II and III), and also network-forming collagen in the basement membrane, type IV collagen, is also widely present in different organs [1]. While both DDRs recognize fibrillar collagens, type IV collagen is recognized only by DDR1 but not by DDR2 [4]. DDR-binding sites in homo-trimeric collagens have been identified by Leitinger's group using triple helical peptide library [6, 7]. The studies identified GVMGFO (O is hydroxyproline) as a high-affinity binding motif for both DDR1 and DDR2 that is present in collagen types I-III. Interestingly GVMGFO motif cannot be found in collagen IV, suggesting that DDR1 binds different motif in this collagen [6]. Although both DDRs recognize the same motif, surrounding sequences seem to influence DDR1 binding and DDR2 seems to have relaxed specificity. For instance, DDR1 has only one binding site in collagen III. While DDR2 binds at least four high-affinity binding sites within the collagen [6].

4. Regulation of ddr signaling: Ectodomain shedding

Most of RTK family proteins bind to soluble ligands for their activation. Their signal activation is determined by the availability of a specific ligand to each RTK, and once the ligand binds to the ectodomain of RTK, they can be endocytosed for signaling, followed by termination of the process. The level of signaling is dependent on the local concentration of these bioavailable ligands, and it can be

regulated by their gene expression or liberation of ligands from the local ECM by proteolysis. On the other hand, ligands of DDRs, collagens, are present in the pericellular spaces as a solid matrix. Therefore, receptor activation processes found in other RTK cannot be applied to DDRs due to following problem: (i) ligand is abundantly present in extracellular space; and (ii) when DDRs bind to collagen in solid ECM, it cannot be dissociated or endocytosed to terminate DDR signaling. It was found that DDR1 employs a unique mechanism to regulate collagen signaling: ectodomain shedding. Significant shedding of DDR1 has been detected upon collagen stimulation of cells [8]. It was found that a disintegrin and metalloprotease 10 (ADAM10) is the responsible enzyme for this collagen-induced DDR1 shedding, and ADAM10-dependent DDR1 shedding plays important roles in termination of DDR1 signaling [9]. The shedding-resistant mutant has longer signaling retention time, suggesting that ADAM10-dependent DDR1 shedding is an important regulatory mechanism of DDR1-mediated collagen signaling. It was shown that DDR1 and ADAM10 form a stable complex even before collagen stimulation without cleavage, and collagen binding to DS domain of DDR1 somehow renders DDR1 available for ADAM10 to cleave [9]. As DDR1 binds to collagen matrix, it also acts as an adhesion molecule. However, for an adhesion molecule to support cell migration, dissociation is as important as adhesion to ECM molecules. ADAM10-dependent shedding seems to be a major mechanism to dissociate DDR1-dependent cell adhesion to promote cell migration since inhibition of the shedding or expression of a shedding-resistant DDR1 mutant significantly inhibited cell migration on the collagen matrix [9]. DDR1 is also spontaneously shed in low level, but responsible shedding enzyme and biological significance have not been identified yet. Although it was reported that ectopic expression of membrane-type 1 matrix metalloproteinase (MT1-MMP) could result in the constitutive shedding of DDR1, endogenous MT1-MMP was not shown to do this [10], and ADAM10 has been ruled out [9]. Shedding of DDR2, on the other hand, has not been described, and MT1-MMP did not shed even with ectopic expression [10].

5. Role of ddrs in cell migration

Both DDRs has been shown to modulate capability of cells to migrate. For DDR1, there are some conflicting reports showing that expression of DDR1 impacts cell migration/invasion negatively and positively. Wang et al. [11] reported that ectopic expression of DDR1a and DDR1b in either MDCK epithelial cells or NIH-3T3 fibroblasts inhibited collagen-stimulated $\alpha 2\beta 1$ integrin-mediated cell migration by inhibiting Stat1/3 tyrosine phosphorylation. The same group subsequently reported

that ectopic expression of either DDR1a or DDR1b in MDCK cells inhibited tubulogenesis in 3D-collagen matrix as well, while expression of the cytoplasmic domain-deleted dominant negative form of DDR1 did not. A proposed mechanism is through the interaction of DDR1 with SHP-2, that counteracts $\alpha_2\beta_1$ -integrin-mediated phosphorylation of STAT3, leading to inhibition of migration and tubulogenesis of MDCK cells [12]. In contrast, using smooth muscle cells derived from DDR1 null mice, it was shown that DDR1 plays a role in collagen-induced cell migration [13], and a similar finding was also reported for squamous cell carcinoma cells that ectopic expression of DDR1a stimulated cell migration on the collagen matrix [9]. T-cell migration through 3D collagen matrix was shown to be inhibited by addition of soluble DDR1-Fc fusion protein, suggesting that engagement of DDR1 to the collagen plays a role in T-cell migration [14]. Similar data were also reported in Th17 T-cells where either knockdown of DDR1 or addition of DDR1-Fc fusion protein inhibited cell migration in 3D collagen [15]. There are some reports showing that DDR1a and DDR1b have different effects on cell migration. In THP-1 human monocytic cells, ectopic expression of DDR1a significantly increased MCP-1 induced cell migration through collagen matrix, but the expression of DDR1b did not influence the cell migration [16]. Similar data was also reported on glioma cells where ectopic expression of DDR1a stimulated both cell migration and invasion while DDR1b expression did not influence [17]. In non-small lung cancer cells ectopic expression DDR1a but not DDR1b enhanced cell migration in the absence of collagen, however, both DDR1a and DDR1b promoted cell migration in a significant manner upon collagen stimulation [18]. A potential reason for the different effects and role of DDR1 and its variants may be cell-type specific or different experimental conditions, but difference upon ectopic expression of DDR1 may potentially be explained by the expression level of ADAM10 as a DDR1-sheddase in different cells, since DDR1 expression without efficient shedding of DDR1 ectodomain inhibits cell migration [9]. Nevertheless, endogenously expressed DDR1 seems to play a role in cell migration and invasion [13, 15].

DDR2 was also shown to support cell migration. It was reported that efficient migration of skin fibroblasts through Matrigel requires DDR2 as DDR2 null fibroblasts showed significantly reduced cell migration compared to cells from heterozygous mice, while expression of DDR2 in the knockout cells recovered their cell migration phenotype [19]. It was also reported that vascular smooth muscle cell migration induced by the hypoxic condition is due to increased DDR2 expression through the signal via p38 MAPK [20].

6. Role of ddrs as cell adhesion molecules

Cell migration requires repetition of cell adhesion to and cell detachment from their ECM substratum. DDRs mediate cell adhesion to collagen, and this may be one of the ways for DDRs to stimulate cell motility. It has been shown for DDR1 that detachment of DDR1-dependent cell adhesion can be carried out by ectodomain shedding by ADAM10 [9]. On the other hand, the well recognised group of cell adhesion molecules is integrins, and collagen binding integrins including $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_{10}\beta_1$ and $\alpha_{11}\beta_1$ are considered to play a role in cell migration on collagenous matrix [3]. Since DDRs and integrins are co-expressed in the cells, a cross-talk between DDRs and integrins is considered to be crucial for cell migration in collagenous environment. It was shown that DDR1 activation by collagen is independent from β_1 integrin activation [21]. However, Xu et al. [22] have reported that activation of both DDR1 and DDR2 promotes $\alpha_1\beta_1$ - and $\alpha_2\beta_1$ -integrins mediated cell adhesion to collagen in 293 cells. This may partially explain proadhesive and promigratory effect of DDRs that has been described in different cell types [9, 13, 15–18]. Whether different promigratory effect in DDR1a and DDR1b can be explained by integrin cross talk still need to be addressed in future, and detailed molecular links between two collagen receptors are to be further investigated. On the contrary in MDCK epithelial cells and NIH3T3 cells DDR1 was found to inhibit cell migration by inhibiting $\alpha_2\beta_1$ integrin-mediated Stat1/3 tyrosine phosphorylation [11]. It is not clear if this discrepancy is due to different cellular context or experimental procedures, but it needs to be further clarified in future.

Recently it was reported that binding of $\alpha_1\beta_1$, $\alpha_2\beta_1$, and $\alpha_{10}\beta_1$ integrins to cartilage matrix depend on non-collagenous surface macromolecules rather than fibrillar collagens [23]. They confirmed that these β_1 integrins could bind to monomeric collagens in Mg^{2+} -dependent manner, but failed to bind to fibrillar collagens. They also have shown that chondrocyte adhesion to fibrillar collagen was significantly inhibited by addition of DDR2 ectodomain, suggesting that role of DDRs as cell adhesion molecules in relative to integrins may be more significant within collagenous microenvironment. It is important to further investigate relative roles of DDRs and integrins as a cell adhesion molecules.

7. Modulation of matrix metalloproteinase expression and activity

Cell migration within 3D ECM environment can be classified into mesenchymal migration and amoeboid migration [24, 25]. In mesenchymal migration, cells degrade

Table 1. A list of reports showing roles of DDRs in MMPs production. A list of references showing DDRs-mediated MMPs upregulation are shown. Upregulated MMPs, references, cell types and requirement of collagen stimulation for MMPs upregulation are shown.

DDR	Upregulated MMPs	References	Cell types	Requirement of collagen
DDR1	MMP-1 MMP-2 & MMP-9	Ferri et al. ²⁸	human smooth muscle cells	No
		Hou et al. ³⁰	mouse smooth muscle cells	No
		Ram et al. ¹⁷	human glioma cells	No
		Park et al. ³¹	human hepatocellular carcinoma	No
		Castro-Sanchez et al. ³²	MDA-MB231, ZR-75	Yes
DDR2	MMP-7 MMP-1	Roberts et al. ²⁹	human smooth muscle cells	ND
		Vogel et al. ²⁷	HT1080	Yes
	MMP-13	Ferri et al. ²⁸	human smooth muscle cells	No
		Xu et al. ³⁷	human chondrocytes	No
		Sunk et al. ³⁸	human chondrocytes	Yes
		Vonk et al. ³⁹	human chondrocytes	Yes
		Majkowska et al. ⁴⁵	human skin fibroblasts	Yes
MMP-14		human rheumatoid synovial cells	Yes	

*not determined

barrier ECM by proteinases to make a path, while in amoeboid migration cells migrate through ECM gaps by squeezing cell body without degrading ECM [24, 25]. The migration mode is dependent on the size of the gaps within ECM and ability of different cells to squeeze the cell body [25, 26]. It is considered that cancer, epithelial and stromal cells migrate through mesenchymal migration and leukocytes employ amoeboid migration [25], but this is likely to be determined by how squeezable the cell bodies are. Nevertheless, when migratory cells face to the barrier matrix that cells cannot migrate through without enlarging the gap, ECM degradation becomes an important step in migration, and role of DDRs in promoting cellular invasion is partially due to modulating expression and activity of matrix metalloproteinases (MMPs), a group of the enzymes that degrades ECM components (Table 1). Vogel et al. [27] first demonstrated that MMP-1 (interstitial collagenase) is upregulated with collagen stimulation through DDR2 in HT1080 human fibrosarcoma cell line. Ferri et al. [28] also reported that ectopic expression of DDR1 and DDR2 upregulated MMP-1 expression. Robert et al. [29] reported that DDR1 knock down downregulated MMP-7 production in human bronchial epithelial cells. It has also been reported that expression of DDR1 upregulates MMP-2 and MMP-9 secretion in different cells. Hou et al. [30] reported that DDR1 expression mediates MMP-2 and MMP-9 expression in mouse smooth muscle cell. Ram et al. [17] described that ectopic expression of DDR1a and DDR1b induced upregulated production of both proMMP-2 and proMMP-9, and collagen stimulation induced activation of proMMP-2 in the cells that overexpress DDR1a but not DDR1b or endogenous DDR1. A similar report was made by Park et al. [31], showing that ectopic expression of DDR1a or DDR1b upregulated proMMP-2 and proMMP-9 production in hepatocarcinoma cell line. Castro-Sanchez et al. [32] described

that stimulation of DDR1 in breast cancer cells, MDA-MB-231 and ZR-75 cells, with type IV collagen have resulted expression of both proMMP-2 and proMMP-9, and this required protein kinase C, activation of epidermal growth factor receptor, production of arachidonic acid (AA) and AA metabolites.

There is a clear link of DDR2 with a human disease, osteoarthritis (OA), which is the most common arthritis displaying an age-dependent cartilage degradation accompanied by loss of joint function. Cartilage is made of small number of chondrocytes and large part of ECM components of type II collagen (45%), aggrecan proteoglycan (45%) and other minor components. During OA development both aggrecan and collagen are degraded by metalloproteinases belonging to ADAMTS (a disintegrin and metalloproteinase with thrombospondin motif) enzymes and MMPs produced from chondrocytes [33]. MMP-13 (collagenase 3) has been implicated in cartilage degradation in OA [34-36], and it has been reported that DDR2 is a receptor that mediates expression of MMP-13 in human chondrocytes [37-39]. Heterozygote DDR2 null mice were shown to be partially protected from experimental OA in mouse accompanied by a decreased level of MMP-13 expression [40], suggesting that DDR2-MMP-13 axis contributes to OA development. However, chondrocytes in cartilage are segregated from fibrillar collagen II in a territorial matrix in cartilage due to the pericellular matrix surrounding chondrocyte. Further investigation is needed to understand the events that allow chondrocyte DDR2 to recognize collagen II in territorial matrix during OA development.

DDR2 may also be related to another form of arthritis, rheumatoid arthritis (RA). RA is an autoimmune inflammatory disease characterized by degradation joint tissue by inflamed synovial pannus tissues. There are two collagenolytic enzymes implicated in cartilage degradation in RA, namely MMP-13 and MT1-MMP both expressed by RA synovial cells. Su et al. [41] reported that DDR2

mediates type II collagen-induced MMP-13 expression. Since MMP-13 was shown to highly expressed at pannus-cartilage junctions [42], it is possible that DDR2-mediated cartilage collagen signal is responsible for this. At the pannus-cartilage junction in RA joints, MT1-MMP was also shown to be highly expressed and responsible to degrade cartilage tissue [24, 43, 44]. Like MMP-13, DDR2 also mediates collagen-induced activation of MT1-MMP gene expression and the function in human fibroblasts and RA synovial cells [45]. Knocking down DDR2 or pharmacological inhibition of DDR2 kinase effectively inhibited collagen-induced MT1-MMP activity including proMMP-2 activation and cellular degradation of collagen, suggesting that cartilage collagen signaling through DDR2 may be an endogenous pathway for MT1-MMP upregulation in RA as well. Since MT1-MMP is an endogenous activator of proMMP-13 [46], upregulation of both MMP-13 and MT1-MMP at the pannus-cartilage junction may play an important role in the progression of RA.

It is known that collagen stimulation also induces MT1-MMP activation in some cancer cells, but neither DDR1 nor DDR2 signaling plays a role in HT1080 fibrosarcoma cells since DDR kinase inhibition did not suppress collagen-induced MT1-MMP activities. In MDA-MB-231 cells, type I collagen did not influence the expression of MT1-MMP, MMP-2, and MMP-9 [45]. These findings suggest that DDR2 signaling may be cell-type specific, and there is another pathway that mediates collagen-induced activation of MT1-MMP.

8. Role of ddr1 in the formation of invadosome membrane structure

When cells invade into ECM, they extend specialized membrane protrusions into ECM that contain a proteolytic enzyme to make a path for migration. These membrane structures are called invadosomes which is rich in actin, and MT1-MMP is the enzyme that is concentrated in these membrane structures [47]. Invadosomes include podosomes that normal cells display and invadopodia that transformed cells extend. They are both thin (0.4–5 μm) and relatively short (1–8 μm) extending into ECM attached basal side of the plasma membrane [47]. Recently it was found that endothelial cells can display peculiar linear actin-rich membrane structure upon culturing cells on collagen fibrils, which occurs along collagen fibrils, named linear invadosomes [48]. Like invadopodia and podosomes, it is rich in actin, cortactin, Tks5, and MT1-MMP [48]. It was then found that formation of the linear invadosomes in MDA-MB231 breast cancer cells is DDR1-dependent [49]. Interestingly DDR1 kinase activity is not required

for this, but it is via Cdc42 small GTPase and its guanine nucleotide-exchange factor, TUBA [49]. Presumably binding of DDR1 to the collagen triggers local activation of Cdc42 to form this invadosome, which in turn contribute to cellular invasion into collagen-rich ECM. However, detailed molecular mechanism how DDR1 triggers this event and *in vivo* role of DDR1-mediated invadosome formation are still to be investigated.

9. DDRs as microenvironment sensors

As described above, binding sites of DDR1 and DDR2 in type II and III collagens have been identified [6, 7], and the precise atomic interaction has been elucidated by crystallographic studies [50]. However, based on the fibrillar structure of collagen solved by X-ray fiber diffraction analysis [51], the DDR binding sites may not be readily exposed on the surface of collagen fibrils. Flynn et al. [52] showed that *in vitro* collagen fibrillogenesis was inhibited in the presence of the soluble ectodomain of DDR1 or DDR2, supporting the notion that DDR binding sites in monomeric collagen are located inside the fibril structure and not readily accessible. It is, therefore, possible that collagen molecules are capable of stimulating DDR only when newly synthesized, but before forming a highly-ordered fibril structure, or when the collagen fibrils are proteolytically, chemically or mechanically damaged to expose DDR-binding sites. One of the high-affinity binding sites for DDR2 in type-II collagen is covered by telopeptide lesion of collagen. Thus proteinases that are capable of removing this part of collagen may be able to convert non-stimulus collagen fibrils into stimulator for DDRs. Indeed, Majkowska et al. [45] reported that while acid-extracted type I collagen fibril with intact telopeptide only moderately induced DDR2-mediated MT1-MMP activation in synovial fibroblasts, the pepsin-treated type I collagen fibrils, whose telopeptide was removed, effectively induced MT1-MMP activation. Furthermore, it was found that culturing rheumatoid synovial fibroblasts on intact cartilage stimulated MT1-MMP activation only moderately, while culturing on trypsin-treated partially damaged cartilage, whose aggrecan and other minor components are removed, stimulated MT1-MMP activation effectively [45]. These findings suggest that DDRs may not be activated by collagen within the intact and healthy tissue, and local proteolytic damages may need to occur to expose DDR binding sites. In other words, DDRs are receptors that detect changes in ECM microenvironment (Figure 1). DDR2 activation results in transcriptional activation of various MMPs as discussed above and detection of

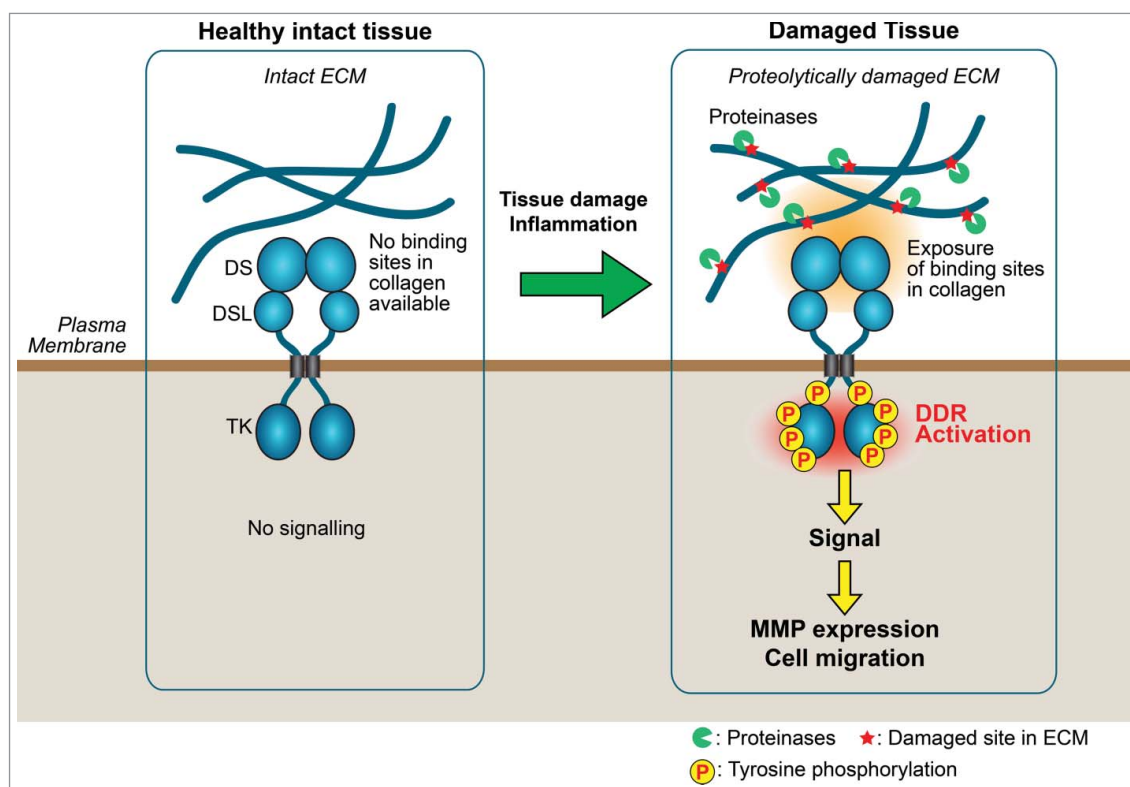


Figure 1. A model of microenvironment sensing by DDRs. When tissue is intact and healthy, DDR binding sites in collagen fibrils in tissue are not readily available for DDRs, thus DDRs cannot bind to collagens and the receptor activation does not occur. Upon tissue injuries causing proteolytic damage of ECM microenvironment, DDR binding sites within collagen become exposed, DDR activation takes place, and induce MMP expression and cell migration. This event may lead to tissue repair or contribute to further tissue damage and promote progression of diseases. DS, discoidin domain; DSL, discoidin-like domain; TK, tyrosine kinase domain.

collagen by DDR2 in partially damaged tissue may play a role in triggering tissue remodeling and repair process by upregulating ECM degrading enzymes.

10. Conclusions

DDR2s are relatively under-investigated RTK, and unique among RTK super-family in its regulation, ligand property and being able to sense changes in ECM microenvironment as discussed above. DDRs are shown to involve in different cancers and arthritis, hence suggested that both DDRs are potential molecular targets for different diseases. There are several efforts being made to develop selective DDR kinase inhibitors, and there are some highly selective DDR1 inhibitors already developed [53-55]. However, pathophysiological roles of DDRs are still to be further revealed before applying DDR inhibitor drug to the clinic. For instance, an aspect of microenvironment sensor and their role in cell migration may be important in both physiological and pathological events, and benefits and detrimental effects upon inhibition need to be defined. Further detailed

investigations of DDRs would contribute to potential novel means to full fill current unmet needs in future.

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