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Review

Sensing extracellular matrix: An update on discoidin domain receptor function

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Abstract

Discoidin Domain Receptors (DDRs) have recently emerged as non-integrin-type receptors for collagen. The two mammalian gene products Discoidin Domain Receptor 1 and -2 constitute a subfamily of tyrosine kinase receptors that are selectively expressed in a number of different cell types and organs. Upon collagen activation, DDRs regulate cell adhesion, proliferation and extracellular matrix remodeling. Here we review the various signaling pathways and cellular responses evoked by activated DDRs. Additionally, we give an overview of the more recent advances in understanding the role of DDRs in various human diseases, in particular during tumor progression, atherosclerosis, inflammation and tissue fibrosis. Furthermore, we discuss potential roles of genes homologous to mammalian DDRs identified in flies, worms and sponges. We show that the structural organization of these DDR-related genes is highly conserved throughout evolution suggesting that invertebrate DDRs may also function as receptors for collagen. By highlighting current questions about these unusual collagen receptors, we hope to attract new research on DDRs from a variety of different fields.

Keywords: Extracellular matrix; Collagen; Tyrosine kinase; Discoidin domain; Receptor signaling; Molecular evolution

Contents

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1. Introduction

Living cells must integrate a myriad of extracellular stimuli into highly cohesive responses. To manage this wealth of information, a diverse array of specialized cell surface receptors exists that binds extrinsic factors such as mitogens, differentiation factors, cell membrane-bound molecules or extracellular matrix (ECM) proteins, and then transmit signals through the plasma membrane. Many of these receptors belong to the family of receptor tyrosine kinases (RTKs) characterized by an extracellular ligand binding domain, a single transmembrane domain and a catalytic tyrosine kinase domain. RTKs have been grouped into 18 subfamilies according to the domain structure of their extracellular region, which defines ligand specificity [1]. This review focuses on one subfamily of RTKs, the Discoidin Domain Receptors (DDRs). Two members of this subfamily are present in the human genome, DDR1 and DDR2. DDRs are unique due to their ligand-specificity and remarkable conservation throughout evolution.

1.1. Being different — DDRs are a variation of the classical tyrosine kinase receptors

Unlike most other RTKs, DDRs are not activated by soluble growth factors. Instead, various types of collagen act as ligands for DDRs. DDR1 is activated by all collagens tested so far, including collagens type I to type VI and type VIII, while DDR2 is only activated by fibrillar collagens, in particular collagens type I and type III [2,3]. DDRs are activated only when collagen is in its native, triple-helical form, as heat-denatured collagen (gelatin), which lacks triple-helical structure, fails to induce kinase activity. While most other RTKs are fully activated in minutes, maximal activation of DDRs occurs several hours after the initial stimulation with collagen [2]. Some attempts have been made to further define the molecular interaction between collagen and DDRs, but the precise location of the DDR-binding site within triple-helical collagen is yet unknown. Recent work however suggested the second quarter of type II collagen has been as a possible binding site for DDR2 [4,5].

Four integrin receptors, formed between the β1 subunit and the α1, α2, α10 or α11 subunit, also act as functional collagen receptors, but do not require DDRs as co-receptors [6]. Conversely, binding of collagen to integrins results in non-DDR-dependent tyrosine phosphorylation events, which are mainly driven by integrin-associated kinases of the Src- and Fak-family. An important and well-described outcome of integrin activation is the alteration in cytoskeletal tension and cell migration, which is mediated by the actomyosin network. In contrast to the integrins, a potential role of DDRs in transmitting these kinds of mechanical stimuli within or between cells has not been explored.

Structurally, DDRs are distinguished from other RTKs by a discoidin domain, an approximately 160 amino acid long homology region first identified in the protein discoidin I from the slime mold *Dictyostelium discoideum*, where it functions as galactose-binding lectin [7–9]. Aside from DDRs, the discoidin I-homology repeat is also present in more than a dozen other mammalian transmembrane as well as secreted proteins. Utilizing the crystal structures of the discoidin domains found in the coagulation factors V and VIII, molecular models of the domains in DDR1 and DDR2 were generated [10,11]. Hallmark of these models is a central eight-stranded beta-barrel, which is stabilized by two intramolecular disulfide bridges, and four finger-like loops protruding from one side of the beta-barrel. The position of these loops is well conserved between discoidin domains of DDRs, blood clotting factors V and VIII or neuropilin [12]. Work with a recombinant preparation of the DDR1 discoidin domain led to the identification of loops 1 and 3 being essential for collagen binding and receptor activation [11]. However, one will have to await a detailed structural analysis of DDRs to draw more definitive conclusions on the architecture of the ligand-binding pocket.

In several cell lines and tissues, DDR1 is partially processed into a 62 kDa membrane-anchored beta-subunit and a 54 kDa soluble extracellular domain-containing alpha-subunit [13]. This process, also termed shedding, is significantly enhanced upon DDR1 activation. Proteases belonging to the ADAM or MT-MMP family could potentially be responsible for DDR1 shedding, since they have been pinpointed as sheddases for a number of other receptors involved in cell-adhesion, including Eph receptors, selectins and the heparin-binding epidermal growth factor [14,15]. In near future, more experimental work will hopefully "shed" better light on the mechanism of DDR1 processing.

Compared to other RTKs, the juxtamembrane regions of DDR1 and DDR2 are much longer (176 and 147 amino acids, respectively). As observed for members of the platelet derived growth factor receptor or Eph receptor subfamilies, we speculate that the juxtamembrane region of DDRs also has an autoinhibitory function [16]. For Eph receptors, it was found that sequences within the juxtamembrane region block the ATP binding site in the kinase domain and, upon ligand binding, need to be displaced prior to activation of the catalytic function. Potentially, the protracted kinetics of DDR activation are the result of a similar rate-limiting structural re-arrangement in the juxtamembrane region that is necessary to overcome an intrinsic auto-inhibition.

1.2. The complexities of DDR1 isoforms

Thus far, five isoforms of DDR1 have been identified, all of which are generated by alternative splicing in the cytoplasmic region [17]. The longest DDR1 transcript encodes the c-isoform with 919 amino acids. The a- and b-receptor isoforms lack 37 or 6 amino acids in the juxtamembrane or kinase domain respectively [9]. DDR1d and DDR1e are truncated variants that lack either the entire kinase region or parts of the juxtamembrane region and the ATP binding site [17]. In contrast, no isoforms have been identified for DDR2 yet.

The relative expression ratios and the post-translational modifications of the DDR1 a- and b-isoforms appear to be controlled by complex regulatory mechanisms. The DDR1b protein is the predominant isoform expressed during embryogenesis, whereas the a-isoform is commonly found in several human mammary carcinoma cell lines [18]. Furthermore,

DDR1a is the predominant isoform during rat neuronal development, but DDR1b is induced following irradiation of astrocytes [19]. Importantly, the alternatively spliced insert in DDR1b contains the motif LLXNPXY that associates with the phosphotyrosine-binding domain of the ShcA adapter protein upon collagen-induced tyrosine phosphorylation [2]. In contrast, the juxtamembrane region of the a-isoform binds to fibroblast-growth factor receptor substrate-2 and appears to be unique in its ability to trigger the migration and pseudopod extension of leukocytes [20,21]. Most likely, the selective availability of juxtamembrane binding sites is critical for differential downstream responses during DDR1 signaling.

In a three-dimensional (3D) tissue culture model with type I collagen gels, epithelial cells overexpressing DDR1a or -b grew slower and formed fewer branches than the parental cell line [22]. In contrast, a truncated DDR1 that resembles the DDR1d isoform had the opposite effects. Possibly, high levels of full length DDR1 can restrict cell migration and proliferation leading to a transient cell cycle arrest. This notion is supported by recent findings in melanoma cells, which undergo DDR2-mediated transient cell cycle arrest when grown within 3D collagen [23]. We conclude that further work is required to fully understand this putative dichotomy of DDR isoform signaling: promoting cell growth in monolayer culture, versus inhibiting it in a 3D, collagen-rich environment. One possibility is that cells in 3D not only sense the lack of tension provided by a rigid 2D substratum, but also are more receptive to diverse cues from the ECM, such as the formation of neo-epitopes during matrix remodeling. Conceivably, DDR-induced signals are converted into different cellular outcomes depending on the nature of the stimulus by collagens, which could either be acid-solubilized, monomeric collagen coated onto tissue culture plastic versus a thick layer of neutralized, and fiber-associated collagen.

1.3. Downstream routes for DDR-induced signaling

DDR1 and DDR2 have a total of 15 and 13 tyrosine residues in their cytoplasmic regions respectively, which serve as potential phosphorylation sites upon receptor activation by collagen. Using phosphopeptide mapping, 3 major and 5 minor phosphorylation sites were recently identified in DDR1 [24]. Sustained phosphorylation of these tyrosines will potentially allow binding of a number of different Src-homology-2 (SH2)- and phosphotyrosine binding (PTB) domain-containing molecules (Fig. 1). Previously, we showed that the alternatively spliced tyrosine-513 of DDR1b directly associates with the PTB domain of ShcA upon receptor activation [2]. Moreover, Shc was also found tyrosine phosphorylated in several cell lines and tissues, such as monocytes or bronchial lavage cells, presumably by activated DDR1 [25,26]. In macrophages, DDR1 induced ShcA phosphorylation led to activation of the TRAF6 complex, which triggers the p38 mitogen-activated protein kinase and NFκB pathways [26–28]. However, Shc-mediated downstream events may be highly cell-type dependent, since activation of DDR1b in human breast cancer T-47D cells results in strong ShcA binding to the receptor but not in protein phosphorylation [2]. Several other molecules were found to directly interact with DDR1, such as Shp-2, an SH2 domain-containing phosphotyrosine phosphatase, and Nck2, an SH2- and SH3 domain containing adapter protein [24,29]. The binding site for Shp-2 was mapped to tyrosine-740 of DDR1 while binding of the p85 subunit of phosphatidyl-inositol-3 kinase is mediated by tyrosine-881 [30]. To summarize these interactions an overview of the DDR1 and DDR2 signaling events is given in Fig. 1.

In a recent approach, Stat5 was found to be a tissue-specific molecule downstream of activated DDR1 [31]. Specifically, mammary epithelial cells that express DDR1 respond with

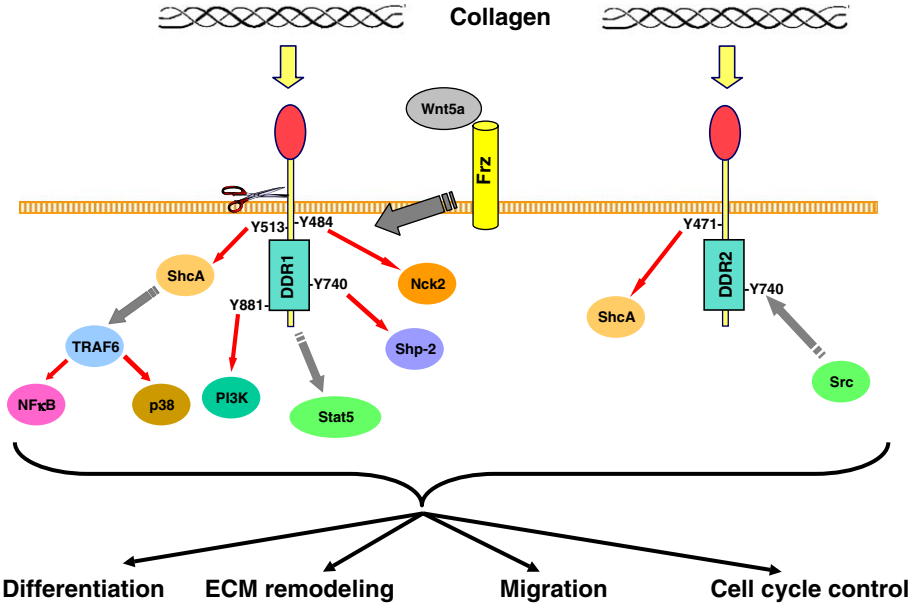


Fig. 1. DDR signaling network. Schematic representation of signaling molecules downstream of DDR1 and DDR2. Red arrows indicate a direct binding, while gray arrows show an indirect interaction. Further details are given in the text.

extended Stat5 activation upon prolactin stimulation compared to control cells. These results suggest that DDR1 plays a pivotal role in maintaining lactation, which is also supported by data from knockout mice (see below). In human breast cancer cells, it was suggested that DDR1 receives lateral input from other transmembrane receptor/ligand complexes, such as Frizzled and Wnt5a, however the integration of these signals within a cellular context requires further investigation [32–34].

In contrast to DDR1, signaling of DDR2 has mainly been studied in hepatic stellate cells and skin fibroblasts. Intriguingly, it was found that full activation of DDR2 by fibrillar collagen requires the presence of ShcA and Src-like tyrosine kinases [35]. Src appears to be an obligate DDR2 partner to allow full autophosphorylation. Like DDR1, ShcA binds to the juxtamembrane region of DDR2 (tyrosine-471), but unlike DDR1, the SH2 domain rather than the PTB domain of ShcA is mediating this interaction. Furthermore, Src-mediated phosphorylation of some DDR2 sites, including tyrosine-740, appears to be auto-inhibitory, at least in a direct binding experiment with purified proteins [36].

Little is yet known about specific transcriptional targets of active DDRs. Using a microarray specifically designed towards matrix genes and their modifiers, it was found that DDR signaling enhances the expression of P-selectin glycoprotein ligand and represses the levels of matrix proteins such as agrin or syndecan-1 [37]. However, large scale arrays and functional protein assays are necessary to connect the signaling routes described above with any specific transcriptional response.

Table 1
Synopsis of DDR1 and DDR2 expression in normal and malignant tissues

	DDR1	DDR2
Normal organ development		
Lung	Bronchial epithelium [41]	
Skin	Keratinocytes [69,70]	Fibroblasts [42]
GI tract	Colon epithelium [17]	
Kidney	Mesangial cells [45], collecting tubules [56]	Early in metanephric mesenchyme [71], in cortical tubules in adult [56]
Heart		Fibroblasts [72,73]
Liver	Hepatitis C virus-associated cirrhosis [74]	Stellate cells [49,57]
Endometrium		Stromal marker [75]
Mammary gland	Ductal elongation and alveolar differentiation [43]	
Cornea	[76]	
Brain	Cerebellum [40]	
Vasculature	Smooth muscle cells [47,54]	
Cancer		
Breast	[8,9,18,32,50]	[36,77]
Ovarian	[78]	
Brain	[79,80]	
Esophagus	[81]	
Lung	[9]	[36]
Immune system	[82]	[83]

2. DDR function in vivo

2.1. Embryonic development

Both DDRs are expressed early during embryonic development. In situ hybridization found DDR1 in the developing neuroectoderm at mouse embryonic day 8 and DDR2 in the nervous system of embryonic rats at day 12 [38,39]. The expression of DDR1 in the developing mouse brain has been studied and found to be highest in oligodendrocytes [84]. Furthermore, overexpression of a dominant-negative form of DDR1 in cerebellar cell and organ cultures caused a strong reduction of neurite outgrowth, suggesting that a collagen-mediated signal is essential for proper tissue development [40]. In the lung, DDR1 appears to be localized to the basolateral surface of the bronchial epithelium, in close contact to the collagen type IV-rich basement membrane [41]. In contrast, DDR2 is a mesenchymally expressed receptor with highest levels in skeletal muscle, skin, kidney and lung tissue [9,42]. A more comprehensive overview of DDR expression patterns is provided in Table 1.

2.2. Insights from the DDR knockouts

The multiplicity of DDR function was further revealed by targeted deletion of either of the two genes in the mouse. Mice lacking DDR1 are viable but much smaller than wild type littermates [43]. Furthermore, a large percentage of knockout females are unable to give birth because developing blastocysts do not implant. Successfully reproducing females are unable to nourish their litters because the mammary gland epithelium fails to secrete milk. Histological analysis showed that the outgrowth of mammary gland ducts in DDR1-null females is delayed during puberty and the terminal end buds are enlarged [43]. A cell-autonomous function of DDR1 mediating these defects was confirmed by transplanting knockout epithelial tissue into wild type recipients [31].

Aberrant morphology and physiology are also seen in the kidney of DDR1-null mice: older knockout animals have proteinurea, which is caused by focal swelling of the glomerular basement membrane accompanied by defects in the slit diaphragm [44]. This might be explained by the fact that mesangial cells isolated from DDR1-null mice displayed a higher proliferative index compared to controls, which was in turn attributed to higher MAPK activation [45]. Although DDR1-null mice have no apparent vascular phenotype, vessel injury of DDR1-null mice identified a crucial function of the receptor controlling vascular smooth cell adhesion, proliferation and MMP production [46,47]. In conclusion, DDR1 appears to be a key regulator of cell morphogenesis, differentiation and proliferation in several organs, including the mammary gland, the vasculature and the kidney.

Consistent with the DDR1 phenotype, mice with a deletion of DDR2 suffer from dwarfism. However, in contrast to the DDR1 phenotype, dwarfism of DDR2 mice is caused by a reduced proliferation rate of chondrocytes [42]. Furthermore, the healing of epidermal wounds, which is normal in the absence of DDR1, is significantly delayed in mice lacking DDR2. This is explained by the fact that skin fibroblasts from

DDR2-null mice are unable to migrate through a reconstituted basement membrane (Matrigel) and show diminished MMP2 activity [48]. As DDR2 is only activated by fibrillar collagens, its role in the accumulation of type I collagen during hepatic fibrosis has been analyzed [49]. In the normal liver, only the mesenchyme-derived stellate cells express DDR2. Cultivation of primary rat stellate cells on type I collagen results in an induction of DDR2 expression and an increase in autophosphorylation, whereas cultivation on type IV collagen-rich Matrigel, which does not function as a ligand, decreased DDR2 abundance. In summary, the generation of DDR knockout mice has been an important step in our understanding of the biological roles of DDR1 and DDR2 in normal tissue function, however more effort is required to identify potentially unique functions in various diseases.

2.3. Cancer

DDRs have been linked to a number of diverse human cancers, through studies of both tumor cell lines and clinical samples. The current body of literature is summarized in Table 1. DDR1 and -2 were first isolated from a breast cancer cell line and a primary colon adenocarcinoma [8,9]. DDR1 was shown to be more highly expressed in cancerous epithelial cells relative to normal epithelial tissue from the same breast tumors [50], and DDR1 and DDR2 transcripts have been shown to have mutually exclusive expression patterns in cancerous epithelial cells and stromal cells respectively [9]. Furthermore, DDR1 has been demonstrated to be a direct transcriptional target of the p53 tumor suppressor gene, highlighting a possible role in carcinogenesis [51]. The inhibition of DDR1 function in cells containing wild type p53 (characteristic of many human cancers) results in increased apoptosis, suggesting DDR1 as a potential target in cancer therapy.

While there are no data as yet showing that DDRs act as transforming oncogenes in carcinogenesis, DDRs likely play a role in the regulation of tumor growth, in particular in the ability of the tumor to metastasize and invade other tissues. It is well documented that the ability of tumor cells to adhere to the extracellular matrix directly affects their invasive potential [52]. The proven interaction of DDRs with the collagenous extracellular matrix therefore implies a regulatory function in tumor cell adhesion and invasion as well as in MMP activity.

2.4. Atherosclerosis

The remodeling of collagens upon vascular injury has been extensively studied [53]. In atherosclerotic tissue from non-human primates fed with a hypercholesterolemic diet, both DDRs were found to be highly expressed by smooth muscle cells within the fibrous cap [54]. The accumulation of collagen, which is a hallmark of atherosclerotic plaque formation, was much less severe in DDR1-null than wild type mice following copper wire injury. Concomitantly, proliferation, migration and MMP2 production of smooth muscle cells isolated from knockout mice were much reduced compared to wild type animals [46,47]. In turn, overexpression of either DDR1 or DDR2 in human smooth muscle cells induced MMP1 as well as MMP2 expression for DDR2 [54]. Furthermore, stretching vascular smooth muscle cells in vitro resulted in upregulation of DDR2 expression, a process dependent on TGF-beta and angiotensin II signaling [55]. It will be of interest to study the precise involvement of DDRs during the progression of atherosclerosis and vascular injury, for example by using genetic mouse models, such as the LDL receptor knockout line.

2.5. Fibrosis of the kidney, liver, lung and skin

The analysis of DDR expression in various human tissues showed highest mRNA levels for both receptors in the adult kidney [9]. To study their role in renal diseases, fibrosis was induced by subtotal nephrectomy, which resulted in upregulation of DDR1 expression [56]. In a complementary approach, treating DDR1-null mice with angiotensin II or a nitric oxide synthase inhibitor induced hypertension to a level similar as in control animals, but resulted in much reduced fibrotic and inflammatory responses (J.-C. Dussaule, submitted for publication). Collectively, these data suggest that DDR1 may be critically involved in the mediation of fibrotic responses in the kidney and that inhibition of DDR1 signaling may represent a potential target to prevent the progression to end-stage renal diseases.

As a consequence of tissue injury by a variety of noxious agents, stellate cells in the liver undergo a transformation from a quiescent to an activated state. As a result, cells turn into highly proliferative myofibroblasts, which secrete large amounts of type I collagen. Work by Olaso et al. showed that the induction of liver fibrosis in an animal model resulted in upregulation of DDR2 specifically in the stellate cells [49]. However, this surge in DDR2 expression is preceded by increased collagen synthesis, suggesting that DDR2 may perpetuate rather than initiate liver fibrosis. Interestingly, in a related study, DDR2 was not only found upregulated in the mesenchymal compartment, but also in biliary epithelial cells from cirrhotic livers, suggesting a much wider tissue distribution and function [57].

In many cases, the etiology of lung fibrosis is poorly understood, leading to the diagnosis of idiopathic pulmonary fibrosis (IPF). A recent study compared CD14-positive cells in the bronchioalveolar lavage fluid obtained from IPF patients with samples from healthy volunteers or from patients with other lung diseases and found significantly higher expression levels of DDR1 in IPF patients [25]. Surprisingly, the expression of the DDR1b-isoform was selectively induced during disease progression. Exposure of lavage cells to collagen or a DDR1-activating antibody increased the expression of MCP-1, interleukins and MMP9. However it remains to be proven that these are direct transcriptional targets of DDR1 and not merely co-regulated with other pro-inflammatory responses. To this end, knockout mice would be an ideal model to validate DDR1 as a target for therapeutic intervention of lung fibrosis.

Excessive deposition of collagenous matrix, termed keloid formation, is a major concern during wound healing. Experiments in rat skin explants showed that DDR1 is upregulated during early fetal development when skin repair occurs without

scar formation, while DDR1 levels are reduced at later stages [58]. It will be important to test whether the various isoforms of DDR1 are differentially expressed during this process, thereby leading to unique signaling events. Although DDR2 levels were unchanged in the explant model, wounding experiments performed with the knockout mice clearly showed that DDR2 plays an equally important role during skin repair [42].

2.6. Inflammation and arthritis

Signaling of DDR receptors in immune cells has only been explored for DDR1. It was found that upon stimulation with lipopolysaccharides or interleukin-1β, monocytes and neutrophils express the a- and b-isoforms of DDR1 [21]. Further studies in a leukemia cell line individually transfected with either of these two isoforms showed that DDR1a promotes adhesion, while DDR1b enhances phorbol ester-induced differentiation into macrophages [26,28]. Additional data suggest that DDR1b is responsible for the upregulation of cytokines such as MIP-1α or MCP-1 during extravasation of macrophages. Potentially, DDR1 is not only involved in pro-inflammatory responses, but also in immune cell maturation per se, since factors derived from the stromal microenvironment, including collagens and other ECM molecules, initiate renewal and differentiation of hematopoietic stem cells [59].

Using a mouse model for arthritis, Xu et al., recently showed that expression of DDR2 and MMP13 is upregulated in knee joints of aged animals [60]. A causative link is supported by data from cultured cells that demonstrate stimulation of DDR2-transfected chondrocytes with type II collagen results in selective induction of MMP-13. Similarly, using the adjuvant-induced rat model for rheumatoid arthritis, an increase of DDR2 expression in synovial cells was reported [61]. In patients with rheumatoid arthritis or osteoarthritis, DDR2 expression was also documented in cells isolated from the synovial fluid, along with high MMP1 activity [62,63].

The association of DDR1 and DDR2 with this wide range of diseases, from cancer to arthritis, serves to illustrate the potential importance of these receptors in human health and disease. Many of these associations are preliminary and to some degree tentative, and more research is required to further elucidate the roles of these unique RTKs in human disease. However, the current data support the concept of DDRs being a molecular sensor that can probe the integrity of the extracellular matrix in a wide variety of conditions. With this notion in mind, DDRs are well positioned to qualify as cell-surface accessible targets for potential therapeutic interventions.

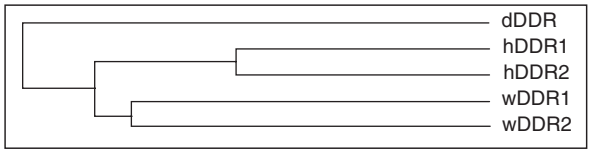
3. Evolution of DDRs

Recent advances in whole-genome sequencing showed that the number of tyrosine kinases has proportionally increased through evolution; whereas yeast shows no sign of tyrosine phosphorylation, the worm and fly genomes reveal about 40 sequences with homology to tyrosine kinases [64]. In comparison, there are 91 tyrosine kinases in the human genome of which 58 are receptor molecules. Surprisingly, the number of DDR-related genes has remained remarkably constant throughout evolution: there are just two genes coding for DDRs in worms (*Caenorhabditis elegans*), fish (*Fugu rubripes*) and humans (Fig. 2A).

The *C. elegans* genome shows two genes with striking homology to mammalian DDRs. They display an identical topology compared to the human homologues, with an N-terminal discoidin domain, a transmembrane region and a C-terminal tyrosine kinase domain. In this review, two genes, annotated as C25F6.4 and F11D5.3 in the *C. elegans* database, are referred to as wDDR1 and wDDR2, as acronyms for the worm homologues of DDRs. An approximately 4.8 kb genomic sequence encodes the 15 exons of wDDR1, which are translated into a 767 amino acid protein. The sequence for wDDR2 comprises 797 amino acids, of which the first 30 amino acids (exon 1) can be absent due to alternative splicing.

A *Drosophila* database search revealed two sequence entries, CG9488 and CG9490, with significant homology to DDRs. Originally, the two entries were assigned to distinct gene

A



B

Discoidin Domain Similarity

	hDDR1	hDDR2	dDDR	wDDR1	wDDR2
hDDR1		88.5%	50.6%	71.3%	78.4%
hDDR2	89.5%		54.1%	73.4%	77.8%
dDDR	75.2%	76.8%		51.3%	53.5%
wDDR1	55.6%	55.6%	59.8%		70.9%
wDDR2	69.9%	73.7%	78.8%	59.2%	

Kinase Domain Similarity

C

	hDDR1	hDDR2	wDDR1	wDDR2
ShcA- PTB ShcA- SH2	LSNPA^{513}Y - - -	- - - ^{471}YDRI	LSNGH^{408}Y - - -	- - -
Grb2-SH2	- - -	^{684Y}TNL	- - -	- - -
Nck- SH2	^{484}YQEP	^{481}YQEP	- - -	- - -
p85- SH2	^{881}YELM	^{825}YKLM	^{594}YSAM	^{718}YSDM
Vav- SH2	^{547}YMEP ^{703}YMEN	^{656}YMEN	- - -	^{595}YMEN
Src- SH2	- - -	- - -	^{675}YEEL	- - -
Shp2- SH2	^{740}YPML	- - -	- - -	- - -
PKA	- - -	RRD^{836}T	- - -	RRP^{771}S

Fig. 2. Relationships amongst human, fly and worm DDRs. (A) Cladogram of human (hDDR), worm (wDDR) and fly (dDDR) homologies. ClustalW (www.ebi.ac.uk/clustalw) was used to generate the cladogram. (B) Similarities of the discoidin or kinase region comparing fly, worm and human sequences. (C) A list of common RTK substrates and their potential interaction sites in the sequence of worm and human DDRs is given.

products, however, a close analysis revealed that both open reading frames, which had been automatically predicted, belonged to a single gene that we abbreviate here as dDDR. CG9488 encodes a 247 amino acid long sequence fragment with homology to the extracellular domain of hDDR2, while the 77 amino acids of CG9490 show homology to the tyrosine kinase domain of other DDRs. Both predicted open reading frames are only 26 kb apart and enclose a third open reading frame coding for 266 amino acids (CG11573), that also shows significant homology to human DDR. To unambiguously reveal the identity of dDDR however, cDNA cloning will be necessary. Intriguingly, a recent survey of *Drosophila* protein kinases revealed a possible role of dDDR in cell cycle progression as gene silencing by RNA-interference resulted in chromosomal abnormalities in fly cells [65].

Genes with tyrosine kinase homology have been described in many other invertebrates including sponges. Using degenerated oligonucleotide primers against the conserved tyrosine kinase domain, PCR cloning revealed 15 different sequences in the freshwater sponge *Ephydratia fluviatilis* [66]. Amongst these sequences, spPTK14 and hyPTK66 show significantly higher similarities to the kinase domain of human DDRs than to any other tyrosine kinase. However, to qualify as DDR-homologues, full-length cloning is required in order to demonstrate that these sponge genes code for transmembrane receptor molecules with an extracellular discoidin domain region. Similarly, the recent completion of the sea squirt *Ciona intestinalis* genome sequence or the expression profiling of the sea urchin *Strongylocentrotus purpuratus* predicted gene products highly similar to DDR1, however a functional analysis is still lacking [67].

The overall organization of all DDR proteins including those from humans, worms and flies, is well conserved. The highest degree of conservation is seen in the catalytic kinase domain. In the case of worms, DDRs exhibit less similarity to one another than to human DDRs (Fig. 2B). Human DDR1 and hDDR2 are 78.0% similar overall, whereas wDDR1 and wDDR2 share only 60.9% similarity. In particular, the kinase domains are more closely conserved in humans than in worms (89.5% versus 59.2%). Interestingly, the kinase and discoidin domains of wDDRs show differing levels of sequence conservation. While the discoidin domains of wDDRs and hDDRs are 71–78% similar, the kinase domain of wDDR1 shares only 55.6% of similar residues with hDDRs. The overall similarity between the wDDR1 and hDDR1 is slightly higher than between wDDR1 and hDDR2, justifying our designation of these genes (Fig. 2B). Although there is currently no experimental proof, it is tempting to speculate that wDDR1 and wDDR2 also recognize collagens as ligands. Remarkably, the diversity of collagen molecules in worms significantly exceeds the diversity in mammals. Potentially due to the absence of calcified structures, the worm cuticle is formed by more than 150 different collagens [68].

Several potential substrate-binding sites can be identified in worm and human DDRs based on consensus motives, however none has been functionally tested in systems other than mammalian cells. The YXXM consensus required for binding of the SH2 domain in the p85 subunit of phosphatidylinositol-3-kinase (PI3-K) is found in all 4 sequences suggesting a common role of PI3-K in human and worm DDR signaling pathways (Fig. 2C). A Vav SH2 domain-binding site is found in all sequences except wDDR1. The consensus site for Nck is found only in human, but not in worm DDRs. Potentially, the Nck binding site may have evolved later, as genes homologous to Nck are present in *Drosophila* but absent in *C. elegans*. Lastly, in the C-terminal tail of both worm and human DDR2 there is a serine/threonine consensus site that may be a potential target for enzymes of the protein kinase A family.

The success of metazoan life relied on the invention of the ECM. This “sticky glue” initiated the development of higher organisms by allowing the construction of a more elaborate architecture within cellular aggregates. Cell adhesion and anchorage receptors probably evolved as essential structural components and facilitators of cell–matrix communication. Collagens are major building blocks in extracellular matrices and are recognized as signaling molecules by two groups of adhesion receptors: integrins and DDR, both of which trigger intracellular tyrosine phosphorylation. Further analysis of proteins from lower invertebrates will demonstrate the degree of functional conservation and evolutionary diversity between different phyla. Particularly, the role of collagen as a signaling molecule will hopefully soon be more widely addressed in non-mammalian systems as well.

4. Future research on DDRs

Over the past decade, work from many laboratories has helped to elucidate the function of DDRs during mammalian development. We now have a good understanding of the dual role of DDRs as collagen sensors: they not only receive outside signals from triple-helical collagen and evoke cellular responses, but are also involved in the regulation of expression of matrix-degrading enzymes, such as MMPs, which are linked to the control and neo-synthesis of ECM molecules.

What lies ahead for research on DDRs? Firstly, we still need to generate the tools to selectively probe DDR function. The production of monoclonal antibodies that specifically bind to the collagen recognition sites within the discoidin domain will be a compelling option. Alternatively, a crystal or NMR structure of a DDR discoidin domain – once available – should facilitate the design of small peptide inhibitors. Based on the slow-kinetics of DDR phosphorylation, we believe that the kinase function is unique among RTKs. Therefore, small molecule inhibitors can be envisioned that are specifically tailored towards the dimerization domain or ATP-binding site within the catalytic region of DDRs.

A second area that deserves further attention is the elucidation of the role of DDRs in cancer and chronic inflammatory diseases. From the examples given in this review, it is clear that DDRs are upregulated in response to many forms of cellular transformation or tissue injury. We need to investigate which transcription factors drive the upregulation of DDRs and how cell- and isoform-specific expression of DDR1 is achieved. Furthermore, most work so far has been performed with type I collagen, thereby neglecting the established role of all other collagens in disease progression. In particular,

collagens other than type I might be equally potent in activating DDRs or might trigger alternative cellular responses. The development of dominant negative receptors or antisense vectors is a first step to test DDR inhibition in tissue culture and animal models. In the coming years, it will be exciting to see whether DDRs will follow the path of other RTKs and mature into well-established clinical targets that have led to a rational therapy of diseases.

5. Note added in proof

The *Drosophila* DDR genomic sequence was recently re-annotated; it is now suggested to encode a 1018-amino acid protein (FlyBase ID: FBgn0053531, Genbank: NP_001014474).

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