The role of the CD44 transmembrane and cytoplasmic domains in co-ordinating adhesive and signalling events

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Summary

CD44 is a widely distributed type I transmembrane glycoprotein and functions as the major hyaluronan receptor on most cell types. Although alternative splicing can produce a large number of different isoforms, they all retain the hyaluronan-binding Link-homology region and a common transmembrane and cytoplasmic domain, which are highly conserved between species. The past decade has seen an extensive investigation of this receptor owing to its importance in mediating cell-cell and cell-matrix interactions in both normal and disease states. Although roles for alternative splicing and variable glycosylation in determining ligand-binding interactions are now well established, the mechanisms by which CD44 integrates structural and signalling events to elicit cellular responses have been less well understood. However, there is now increasing evidence that CD44 is assembled in a regulated manner into membrane-cytoskeletal junctional complexes and, through both direct and indirect interactions, serves to focus downstream signal transduction events.

Key words: CD44, Hyaluronan, ERM, Ezrin, Phosphorylation, Proteolytic cleavage

Introduction

Hyaluronan is a ubiquitous glycosaminoglycan component of the extracellular matrix in vertebrates. It consists of large linear polymers of repeating disaccharide units often exceeding 4 MDa, although smaller chain lengths can arise from degradation in conditions of physiological stress (for reviews, see Noble, 2002; Tammi et al., 2002). CD44 was the first identified transmembrane hyaluronan-binding protein, and interest in this receptor stemmed from the demonstration that CD44-hyaluronan interactions mediate cell adhesion and migration in a variety of physiological and pathophysiological processes, including tumour metastasis, wound healing and leukocyte extravasation at sides of inflammation (for reviews, see Bajorath, 2000; Lesley et al., 1993; Martin et al., 2003; Ponta et al., 2003). More recently, several other transmembrane receptors containing a hyaluronan-binding Link module have been identified as potential hyaladherins (hyaluronan-binding proteins) (reviewed by Day and Prestwich, 2002). In contrast to these receptors, CD44 is widely distributed, which makes it the major hyaluronan receptor on most cell types. Moreover, it has been a consistent observation that CD44 is upregulated on many activated or diseased cells. The functional role of this receptor is now better understood owing to the generation of CD44-null mice and the loss-of-function studies such mice have enabled (reviewed by Ponta et al., 2003). The most striking observations to come from these animals is that they develop normally and, if left unchallenged, show few phenotypic abnormalities. However, in models of inflammatory disease or in tumour-prone mice, the effects of CD44 deletion are striking, indicating that the main role of CD44 may be in the response to environmental insult and in disease progression.

For an adhesion receptor to mediate its affects, it must not only bind to its extracellular ligand(s) but must also engage the cytoskeleton and co-ordinate signalling events to enable the cell to respond to changes in the environment. In the case of CD44, depending on the cell type, engagement of hyaluronan can result in cell rolling, cell migration or cell chemotaxis, as well as in hyaluronan internalization or assembly of a hyaluronan-rich pericellular matrix. In turn, these events can modulate cell proliferation, cell survival and differentiation, and remodelling of the extracellular matrix. Moreover, it is clear from these studies that CD44, and in particular the alternatively spliced CD44 isoforms, has roles that are independent of hyaluronan binding. Given this plethora of cell-type-dependent responses, it has proved difficult to define specific pathways downstream of CD44. However, a clearer picture of the mechanism by which CD44 interacts with the intracellular machinery is emerging, and we now have clues as to how these events might underpin aspects of disease progression. Here, we particularly focus on the roles of the transmembrane and cytoplasmic domains in these processes.

Structure and sequence conservation of CD44

A single gene encodes CD44 (Fig. 1A); it has 20 exons, from which a large number of protein products are generated as a result of extensive alternative splicing. The most abundant form of CD44, standard CD44 (CD44s), consists of an N-terminal signal sequence (exon 1), a Link-homology hyaluronan-binding module (exons 2 and 3), a stem region (exons 4, 5, 16 and 17), a single-pass transmembrane domain

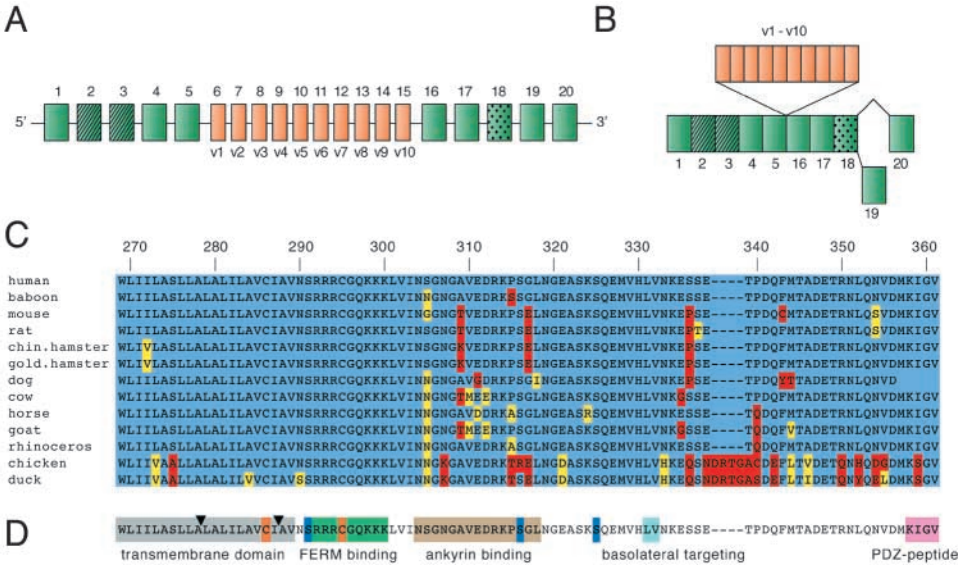

Fig. 1. CD44 structure and sequence conservation. (A) Genomic organization of CD44. The human gene encoding CD44 consists of 20 exons and is located at 11p13. The exons encoding the hyaluronan-binding domain and transmembrane domain are cross-hatched and stippled, respectively. (B) mRNA splicing patterns in CD44. The standard form of CD44, CD44s, comprises exons 1-5, 16-18 and 20 (green). Most variant forms of CD44, CD44v, contain the standard exons with combinations of exons 6-15 (v1-v10) (orange). The inclusion of exon 19, normally absent in most CD44 transcripts, results in a CD44 short-tail variant owing to use of an alternative translation stop codon. (C) Cross-species sequence comparison of the CD44 transmembrane and cytoplasmic domains. Orthologous CD44 sequences (human, P16070; *Papio hamadryas* (baboon), P14745; *Mus musculus* (mouse), P15379; *Rattus norvegicus* (rat), P26051; *Cricetulus griseus* (chinese hamster), P20944; *Mesocricetus auratus* (golden hamster), Q60522; *Canis familiaris* (dog), Q28284; *Bos taurus* (cow), Q29423; *Equus caballus* (horse), Q05078; *Capra hircus*(goat), CD051825; *Ceratotherium simum simum* (rhinoceros), AF045939; *Gallus gallus* (chicken); AAD37443; *Anas platyrhynchos* (duck), AAK40246) were aligned using the ClustalW program (EBI). In reference to the human sequence, the identical, physicochemically conserved and non-conserved amino acid residues are shown in blue, yellow and red, respectively. Note that the dog sequence is incomplete. (D) Sequence features of the human CD44 transmembrane and cytoplasmic domains. The transmembrane domain (grey) contains two sites for regulated intramembranous cleavage (arrowheads). Several functional motifs have been identified including a FERM-binding domain (green), ankyrin-binding domain (brown) and a basolateral targeting motif (light blue). Other posttranslational modifications include phosphorylation on serine residues (blue) with a protein kinase C (PKC) site at Ser291, a Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) site at Ser325, and a predicted protein kinase A (PKA) site at Ser316. Palmitoylation is also known to occur, with prospective sites being Cys286 and Cys295 (orange). The cytoplasmic tail terminates in a potential PDZ-domain-binding peptide (magenta).

(exon 18) and a cytoplasmic domain (exon 20) (Fig. 1B). Alternative splicing of CD44 predominantly involves variable insertion of different combinations of exons 6-15 (also known as variant exons v1-v10) into the stem region, the alternatively spliced isoforms being most commonly expressed in epithelial cells and also upregulated in disease states**.** In addition, nearly all CD44 cDNAs isolated have exon 19 spliced out, producing an open reading frame encoding a 73 amino acid cytoplasmic domain. The inclusion of exon 19 would generate a short 5 amino acid cytoplasmic tail terminating at Arg294 (Goldstein et al., 1989) (Fig. 1B). Little attention has been paid to isoforms that included exon 19, because RT-PCR studies revealed such transcripts are of very low abundance compared with the ‘long-tail’ isoform (Goldstein and Butcher, 1990). However, more recently, ‘short-tail’ isoforms have been detected at reasonable levels in chondrocytes (Jiang et al., 2001).

A recent search of the DNA databases revealed CD44 orthologues from eleven mammalian and two avian species, and these share an overall 47-93% amino acid identity relative with the human sequence. The regions sharing most identity are the Link, transmembrane and cytoplasmic domains. Alignment of these sequences shows that the transmembrane domain is essentially invariant. The cytoplasmic domain is also highly conserved except for a small insertion towards the C-terminus in the aves (Fig. 1C). This high degree of sequence conservation predicts that transmembrane and cytoplasmic domains are crucial for CD44 function. Accordingly, several post-translational modification sites and protein interaction domains have been mapped (Fig. 1D). We discuss these below.

Post-translational modifications of the CD44 transmembrane and cytoplasmic domains

Phosphorylation

In cultured cells, CD44 is constitutively phosphorylated at Ser325 in the cytoplasmic tail (Fig. 1D) (Neame and Isacke, 1992; Peck and Isacke, 1998; Pure et al., 1995). Ser325 phosphorylation is estimated to occur on ~25-40% of CD44 molecules and is mediated, at least in part, by Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) (Lewis et al., 2001). The role of Ser325 phosphorylation remains poorly defined, although mutations at this site impair hyaluronan-mediated cell migration (Peck and Isacke, 1996; Peck and Isacke, 1998). What is clear is that CD44 phosphorylation is dynamically regulated in that activation of protein kinase C (PKC) by phorbol esters results in a novel form of phosphorylation switching. Total levels of CD44 phosphorylation are not significantly altered after PKC activation, but there is an almost complete dephosphorylation at Ser325 accompanied by a concomitant phosphorylation at alternative serine(s) (Legg et al., 2002). This regulates CD44 function by modulating the interaction with ERM (ezrin, radixin, moesin) proteins (see below). Additionally, the highly conserved Ser316 residue lies in a predicted protein kinase A (PKA) consensus site. Although this site is not phosphorylated in resting cells (Neame and

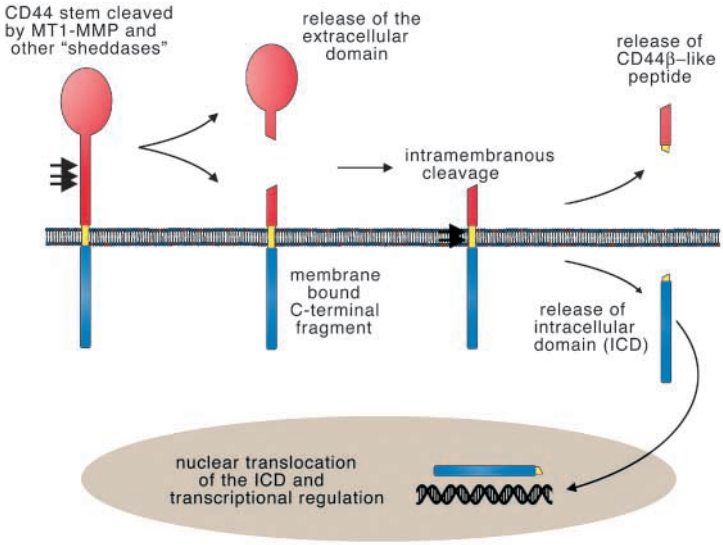

Fig. 2. Proteolytic processing of CD44. The extracellular stem region of CD44 is proteolytically cleaved by MT1- and MT3-MMPs in addition to other CD44 sheddases, leaving a CD44 C-terminal fragment (CTF) embedded in the plasma membrane. The cleaved extracellular region is either secreted into the fluid phase or sequestered to the extracellular matrix. Extracellular domain cleavage is necessary for further processing of the CD44-CTF by regulated intramembranous proteolysis (RIP) by presenilin-1/γ-secretase activity. RIP processing results in the secretion of a CD44β-like peptide and the generation of a CD44 intracellular domain fragment (ICD). The CD44-ICD has been shown to be involved in nuclear signalling.

Isacke, 1992), Ser316 can be phosphorylated after cell stimulation (G. Tzircotis, R.F.T. and C.M.I., unpublished).

Palmitoylation

CD44 is reversibly palmitoylated (Bourguignon et al., 1991; Guo et al., 1994), the prospective acylation sites being Cys286 and/or Cys295 (Fig. 1D). Clearly defined acylation motifs for integral membrane proteins have not been defined, and the roles of this modification in such proteins also remain unclear (reviewed by Bijlmakers and Marsh, 2003). In the case of CD44, acylation has been reported to impair anti-CD3-mediated signal transduction in lymphocytes (Guo et al., 1994) and enhance the association of CD44 with ankyrin (Bourguignon et al., 1991). Given the location of these cysteine residues in the CD44 sequence, palmitoylation might also play a role in partitioning CD44 into membrane subdomains and/or in regulating its association with ERM proteins.

Modification by proteolytic processing

It has long been recognized that the extracellular domain of CD44 is subject to regulated proteolytic cleavage (reviewed by Cichy and Pure, 2003). CD44 cleavage can be blocked by inhibitors of matrix metalloproteinases (MMPs) and ADAMs (a disintegrin and metalloproteinase) (Bazil and Strominger, 1994; Okamoto et al., 1999a; Shi et al., 2001), and membrane-type (MT1)-MMP and MT3-MMP have been shown to release soluble CD44 (Kajita et al., 2001; Mori et al., 2002). However, recent work has revealed that further proteolytic processing occurs within the residual CD44 transmembrane and cytoplasmic domains (Fig. 2). Saya and colleagues demonstrated that CD44 cleavage can generate two cell-associated CD44 species (~25 kDa and ~12 kDa) in addition to the secreted extracellular domain fragment (Murakami et al., 2003; Okamoto et al., 1999a; Okamoto et al., 2001; Okamoto et al., 1999b). The ~25 kDa species corresponds to the residual membrane-bound C-terminal fragment (CTF), whereas the major product isolated from the ~12 kDa band is a CD44 intracellular domain (ICD) fragment resulting from a cleavage just inside the CD44 transmembrane domain (Okamoto et al., 2001) (Fig. 1D). Most recently, presenilin-1/γ-secretase was shown to mediate this, cleaving after Ala280 and Ile287 in the transmembrane domain (Fig. 1D) (Lammich et al., 2002; Murakami et al., 2003). Moreover, the ICD fragment appears to be derived sequentially from the CTF since incubation with membrane-permeable protease inhibitors increased the accumulation of the ~25 kDa band and prevented the appearance of the ~12 kDa band (Okamoto et al., 2001).

The generation of the soluble ICD fragment represents a new example of what has been termed regulated intramembrane proteolysis (RIP) (Brown et al., 2000). Targets of this activity include both Notch and the amyloid precursor protein (APP), and intriguing parallels can be drawn with CD44. Notch and APP are both cleaved by presenilin-1/γ-secretase to generate cytoplasmic fragments that can translocate to the nucleus and promote transcription (Cao and Sudhof, 2001; Steiner and Haass, 2000). Additionally, APP proteolysis also results in the extracellular release of the Aβ peptides that are involved in amyloid plaque generation. Using a dual-epitope-tagged CD44 construct, it was shown that transmembrane cleavage resulted in the secretion of an analogous peptide (Lammich et al., 2002). The function of this CD44β-like peptide is not known, but it might promote the clearance of transmembrane domains from the plasma membrane. Significantly, it has been demonstrated that the released CD44-ICD fragment translocates to the nucleus and stimulates transcription via a phorbol ester response element and that one of its target genes is the gene encoding CD44 itself (Okamoto et al., 2001).

Functional requirement of the transmembrane and cytoplasmic domains

A frequently asked question with respect to adhesion receptors is whether intracellular events can modulate ligand binding. In the case of CD44, it is apparent that cell types differ dramatically in their hyaluronan-binding ability but these differences seem predominantly to reflect variable receptor glycosylation (for reviews, see Kincade et al., 1997; Lesley et al., 1997; Naor et al., 1997). A transmembrane domain and a minimal cytoplasmic domain are required for efficient ligand binding (Lesley et al., 1992; Thomas et al., 1992), probably stabilizing CD44 at the plasma membrane and promoting receptor clustering. However, a specific sequence is not required, because either domain can be replaced with an equivalent domain from unrelated receptors without impairing

or enhancing binding of soluble hyaluronan, adhesion of cells to a hyaluronan substratum or hyaluronan-dependent rolling (Gal et al., 2003; Lesley et al., 2000; Perschl et al., 1995a; Perschl et al., 1995b). Consequently, in in vitro systems, there is no defined role for the CD44 transmembrane and cytoplasmic domains in 'inside-out' signalling. By contrast, there is increasing evidence that these highly conserved domains are required for events downstream of ligand binding.

Hyaluronan internalization

Early studies (Culty et al., 1992) demonstrated that, in macrophages, chondrocytes and transformed fibroblasts, CD44 can mediate uptake of hyaluronan and that a significant proportion of the internalized ligand is degraded within the cell. More recently, increasing evidence indicates that CD44-mediated internalization of hyaluronan and its subsequent degradation are physiologically important (reviewed by Knudson et al., 2002). For example, in transgenic studies where CD44 was downregulated in basal keratinocytes, the resulting skin and corneal lesions were associated with abnormal hyaluronan accumulation (Kaya et al., 1997; Kaya et al., 1999). Similarly, decreased CD44 expression correlates with excess hyaluronan accumulation in CD44-null mice in models of lung injury and inflammation (Teder et al., 2002) and in patients with lichen sclerosus et atrophicus (LSA) (Kaya et al., 2000), solitary cutaneous myxomas (Calikoglu et al., 2002) and myxoid dermatofibroma (Calikoglu et al., 2003).

The mechanism of CD44-hyaluronan internalization is not known but it is clear that the CD44 cytoplasmic domain interacts with components of the cell-trafficking machinery. Within the cytoplasmic domain, a dihydrophobic Leu331/Val332 motif is required for delivery of CD44 to the lateral plasma membrane of polarized epithelial cells (Sheikh and Isacke, 1996) (Fig. 1D). The requirement for a dihydrophobic motif for basolateral targeting has previously been identified in lysosomal integral membrane protein (LIMP)-II, tyrosinase and major histocompatibility complex (MHC) class II invariant chain and, like these, CD44 has an acidic residue in the –4 position relative to the dihydrophobic motif (Heilker et al., 1999). In these other receptors, this dihydrophobic motif also mediates rapid internalization from the plasma membrane by clathrin-dependent endocytosis. However, CD44 appears to be excluded from clathrin-coated pits (Isacke, 1994) and internalizes hyaluronan by a non-clathrin, non-caveolae-dependent mechanism, which is followed by CD44 recycling (Tammi et al., 2001). This suggests that the CD44 cytoplasmic domain contains additional information that prevents recruitment into the coated pits and allows, at least in some cell types, ligand-associated receptor to interact with non-clathrin internalization machinery.

Recent analysis of hyaluronan internalization in primary cultures of articular chondrocytes show that these cells have abundant levels of CD44 transcripts containing exon 19, which results in the expression of the tailless CD44 isoform. Selective downregulation of this isoform results in enhanced hyaluronan internalization and a reduction in the size of the hyaluronan-rich cell-associated matrix, indicating that tailless CD44 acts as a dominant-negative inhibitor of the long-tail isoforms by competing for ligand binding, but lacks the information required for internalization (Jiang et al., 2001).

Interaction with the cytoskeleton

Cell migration, as opposed to cell rolling, requires active rearrangements of the cytoskeleton. In cultured cells, CD44 is strongly localized to the microvilli and regions of actin polymerization, such as membrane ruffles, which suggests that it associates with the actin cytoskeleton. Because the CD44 cytoplasmic domain does not contain any actin-binding sites, this interaction is indirect and mediated by cytoskeleton-associated proteins. The best characterized of these are the ERM proteins and the related protein merlin. These form a subfamily within the band 4.1 superfamily and function as regulatable linkers between transmembrane proteins and the actin cytoskeleton (for reviews, see Bretscher et al., 2002; Gautreau et al., 2002; Mangeat et al., 1999; Turunen et al., 1998). The CD44 binding region for ERM proteins (Legg and Isacke, 1998; Yonemura et al., 1998), merlin (Morrison et al., 2001) and band 4.1 proteins (Yonemura et al., 1998) consists of two clusters of basic amino acids (^{292}RRRCGQKKK^{300}) next to the CD44 transmembrane domain (Fig. 1D). Additionally, ERM proteins can also interact with similar basic regions in other transmembrane receptors, such as members of the intercellular adhesion molecule (ICAM) family (Heiska et al., 1998; Helander et al., 1996; Serrador et al., 1998), CD43 (leukosialin) (Yonemura et al., 1998), syndecan-2 (Granes et al., 2000) and L-selectin (Ivetic et al., 2002).

Structurally, the ERM proteins consist of a ~300 amino acid FERM (band 4.1, ERM) domain present at the N-terminus, which forms a three-lobed cloverleaf structure, followed by an α-helical central region and a C-terminal domain that contains an F-actin-binding site. When the ERM proteins are in their closed, inactive form, the C-terminal domain binds across lobes 2 and 3, blocking both the binding of F-actin to the C-terminal domain and binding of the FERM domain to transmembrane receptors. Activation of ERM proteins is mediated by phosphorylation and binding to membrane phospholipids (for reviews, see Bretscher et al., 2002; Gautreau et al., 2002; Mangeat et al., 1999; Turunen et al., 1998), and structural studies have demonstrated that phospholipids bind to the basic cleft between lobes 1 and 3, releasing the C-terminal domain (Hamada et al., 2000; Pearson et al., 2000). In this open active conformation, the ERM C-terminal domain can associate with actin, whereas transmembrane receptors can bind to a shallow groove in lobe 3 of the FERM domain (Hamada et al., 2003). Activation of the more distantly related ERM family member merlin is also regulated by phosphorylation, but it is the dephosphorylated form of merlin that binds to CD44. The merlin C-terminal domain lacks an actin-binding site and consequently a CD44-merlin complex cannot associate directly with the cytoskeleton (Morrison et al., 2001; Ponta et al., 2003).

This ability of the ERM/merlin proteins to switch between an active and inactive conformation, together with the competition between the ERM proteins and merlin for CD44 binding, provides a mechanism to make and break the CD44-cytoskeletal association. A further level of regulation involves phosphorylation of the CD44 cytoplasmic domain. The PKC-triggered switch from Ser325 phosphorylation to Ser291

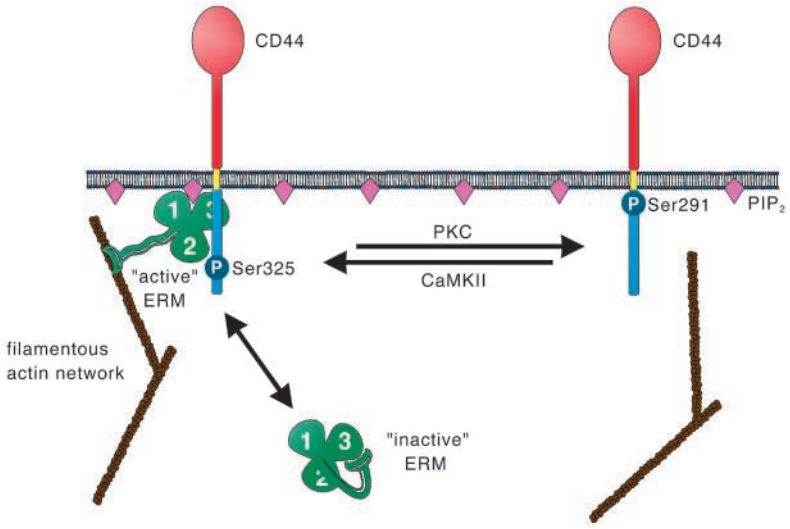

Fig. 3. Model for the regulation of the CD44-ERM complex by dynamic phosphorylation of the CD44 cytoplasmic tail. The ERM (ezrin, radixin, moesin)-family proteins can function to crosslink transmembrane receptors, including CD44, to the cytoskeleton. Their basic structure consists of the three-lobed N-terminal FERM domain followed by a coiled-coil region and a C-terminal domain that contains an F-actin binding site. In their 'inactive' conformation, the C-terminal domain binds to the FERM domain, masking both transmembrane receptors and F-actin interaction sites. Conformational regulation between the 'inactive' and 'active' forms involves complex mechanisms including phosphorylation and binding to the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2) (reviewed by Bretscher et al., 2002). The cytoplasmic tail of CD44 is phosphorylated at Ser325 by CaMKII and this form of the receptor binds to an 'active' ERM protein that links CD44 to the actin cytoskeleton. PKC activation results in a concomitant dephosphorylation of Ser325 and phosphorylation of Ser291, resulting in disengagement of the ERM proteins and loss of cytoskeletal association.

phosphorylation (Legg et al., 2002) (Fig. 1D and Fig. 3) results in the dissociation of ezrin. Moreover, phosphorylation at Ser291 is required for directional migration of cells in a phorbol ester gradient (Legg et al., 2002).

Given the importance of this phosphorylation switching in CD44-mediated chemotaxis, dephosphorylation of these residues is probably tightly controlled. The phosphatases responsible have not been identified, but the presence of a conserved binding site at the CD44 C-terminus for PDZ (PSD-95/Dlg/ZO-1)-domain-containing proteins (Fig. 1D) is of interest. The four terminal amino acids (KIGV in mammals and KSGV in aves; Fig. 1C) conform to the consensus for binding sites for class I and class II PDZ proteins (Hung and Sheng, 2002). Although no specific interaction has been assigned to this motif in CD44, PDZ-binding sites in other receptors, such as the syndecans, have been shown to interact with PDZ-domain adaptor proteins, which in turn can associate with protein phosphatases (reviewed by Bass and Humphries, 2002). Deletion of the syndecan-4 C-terminal amino acids produces a receptor that is hyperphosphorylated in resting cells and exhibits impaired dephosphorylation following stimulation with fibroblast growth factor 2 (Horowitz et al., 2002). How these phosphorylation/dephosphorylation events are regulated in CD44 has yet to be determined but it is clear that hyaluronan, and in particular hyaluronan fragments, are promigratory (for reviews, see Noble, 2002; Toole et al., 2002) and that binding to CD44 can stimulate several downstream signalling pathways (see below), including activation of PKCα (Slevin et al., 2002). Moreover, the ERM proteins themselves act as scaffolding molecules to focus signalling molecules at the cell cortex (Bretscher et al., 2002). For example, ezrin associates with PKC (Ng et al., 2001), thereby providing a potential mechanism by which phosphorylation of CD44 and association with the cytoskeleton can be tightly regulated.

Other mechanisms also modulate association of CD44 with the cytoskeleton or promote cytoskeletal rearrangements. For example, it has been reported that CD44 can directly associate with members of the Rho-family GTPases, their exchange factors and adaptor molecules (reviewed by Turley et al., 2002). The nature of these interactions has yet to be established, but association with RhoA can promote the binding of the membrane-cytoskeleton linker protein ankyrin to a specific motif within the CD44 cytoplasmic domain (Fig. 1D) and this plays a role in hyaluronan-dependent cell migration and anchorage-independent growth (Bourguignon et al., 1999). In addition, the highly conserved transmembrane domain of CD44 (Fig. 1B) is known to partition the receptor into detergent-insoluble glycosphingolipid-enriched plasma membrane domains (lipid rafts) (Neame et al., 1995; Perschl et al., 1995a), and this association has been reported to stabilize the interaction of CD44 with the actin cytoskeleton (Oliferenko et al., 1999). Importantly, there is evidence that the partitioning of CD44 into lipid rafts can be regulated. The proportion of receptor found in these complexes is cell-type dependent (Neame et al., 1995), and CD44 can be displaced from the rafts by expression of E-cadherin, which results in a consequent downregulation of hyaluronan binding and tumour cell invasion (Xu and Yu, 2003). It is tempting to speculate that palmitoylation of the CD44 transmembrane and cytoplasmic tail provides a regulatable mechanism for the association with lipid rafts, although this has yet to be determined experimentally.

Coordination of signalling responses

From early studies it was evident that, in addition to its ability to engage the cytoskeleton, CD44 can also transduce intracellular signalling events leading to alterations in gene expression in response to ligand binding or crosslinking with specific antibodies (for reviews, see Ponta et al., 2003; Pure and Cuff, 2001). Like all of the major classes of adhesion receptor, CD44 lacks intrinsic kinase activity and must therefore associate with other proteins to modulate signalling.

Indeed, many intracellular signalling components form complexes with the CD44 cytoplasmic tail, the most widely reported being Rho-family GTPases and associated molecules (see above) and members of the Src family of non-receptor tyrosine kinases (reviewed by Turley et al., 2002). CD44 co-

immunoprecipitates with Src, Lyn, Lck, Fyn and Hck, and antibody-induced activation of CD44, at least in some cell types, stimulates tyrosine phosphorylation of these kinases and their substrates (Bates et al., 2001; Bourguignon et al., 2001; Ilangumaran et al., 1998; Roscic-Mrkic et al., 2003; Taher et al., 1996). However, as in the case of Rho-family GTPases and associated components, the mechanism of this interaction with CD44 is not known. Src-family kinases are modified by acylation, and these modifications facilitate their targeting to lipid rafts. CD44 is similarly partitioned by its transmembrane domain (see above). Consequently, these components might not interact directly but might instead be co-immunoprecipitated owing to their co-localization in lipid rafts (Ilangumaran et al., 1998).

Currently, stronger evidence for a signalling role for CD44 comes from its ability to act as a co-receptor. In this respect, two distinct but somewhat overlapping categories of co-receptor function have been described. First, CD44 can bind growth factors and cytokines (Bennett et al., 1995; Jones et al., 2000; Roscic-Mrkic et al., 2003; Sherman et al., 1998; Tanaka et al., 1993; Weber et al., 1996) or MMPs that can process growth factors to their active form (reviewed by Isacke and Yarwood, 2002). Thus, CD44 can indirectly promote signalling events by modulating the activity, affinity or localized concentration of signalling factors. Second, CD44 can associate with and modify the function of growth factor receptors and also members of the MT-MMP family. For example, CD44 acts as a co-receptor for the ErbB family of receptor tyrosine kinases and for the c-Met receptor, and these associations are essential for activation of receptor kinase activity and the regulation of diverse cellular processes, including cell survival, proliferation and differentiation (Bourguignon et al., 1997; Orian-Rousseau et al., 2002; Sherman et al., 2000; van der Voort et al., 1999; Yu et al., 2002). These interactions could simply reflect association of the receptor extracellular domains that alters the conformation of the receptor tyrosine kinase, but current evidence indicates a more complex scenario. ErbB4 forms a complex with its ligand, heparin-binding epidermal growth factor (HBEGF), matrilysin (MMP7) and alternatively spliced CD44 isoforms containing the v3 exon (Yu et al., 2002). In this complex, HBEGF precursor bound to heparin sulphate-modified CD44 is cleaved by MMP7 thereby activating ErbB4. It is not known whether the CD44 transmembrane and cytoplasmic domains play a role in this process, although recruitment of CD44 to lipid rafts and/or association of the cytoplasmic domain with the actin cytoskeleton may well be important for promoting heterologous receptor interactions. Indeed, the CD44 cytoplasmic domain and in particular the ERM-binding site is required for the c-Met ligand – scatter factor/hepatocyte growth factor (SF/HGF) – to induce activation of the MEK and Erk kinases downstream of its high-affinity receptor (Orian-Rousseau et al., 2002).

CD44 can also associate on the cell surface with MT1-MMP (Mori et al., 2002) and, as described above, is a target for this protease, which results in cleavage of the CD44 extracellular domain. This cleavage can facilitate cell migration and reorganization of the extracellular matrix (reviewed by Cichy and Pure, 2003) but, in addition, is a prerequisite for further proteolysis of the cell-associated CTF to release the ICD, which can then translocate to the nucleus and regulate gene transcription (Fig. 2) (Okamoto et al., 2001). This form of CD44 signalling has only been shown in vitro thus far, but evidence for its physiological importance comes from the demonstration that CD44 CTFs are upregulated in breast, lung, colon and ovarian carcinomas (Okamoto et al., 2002). Although the range and nature of transcriptional targets for the CD44 ICD has yet to be fully investigated, at least one known target is the gene encoding CD44 itself (Okamoto et al., 2001). Because the newly synthesized CD44 may have altered splicing and glycosylation, this regulated CD44 cleavage and stimulation of intracellular signalling provides a potentially important mechanism for altering the repertoire of CD44 molecules presented at the plasma membrane (Kawano et al., 2000).

Conclusions and perspectives

From its initial identification as a transmembrane hyaluronan receptor mediating cell adhesion and migration, the list of functions ascribed to CD44 has increased annually and includes activities independent of hyaluronan binding. This raises the important question of how and why a single receptor can mediate such diverse cellular processes. Alternative splicing and differential glycosylation of the extracellular domain are important regulators of CD44 function, but these do not provide a full explanation. Rather, in order to elicit these complex cellular responses, CD44 must co-ordinate both structural and signalling events. Many of the precise mechanisms have yet to be clearly elucidated, but the CD44 cytoplasmic domain appears to form a focus for the assembly of both cytoskeletal elements and signalling complexes, often in concert with other plasma membrane receptors. Regulated assembly of these complexes is, at least partly, achieved through post-translational modifications of the CD44 cytoplasmic domain. A major challenge in the future will be to understand the cell-type specificity of these interactions and modifications so that the full complexity of CD44 function within different tissues in both normal and pathological states can be unravelled. Armed with this knowledge, we may be able to target these specific pathways and provide new opportunities for therapeutic intervention.

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