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REVIEW

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CD44: physiological expression of distinct isoforms as evidence for organ-specific metastasis formation

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Abstract Continuous progress has been achieved during recent decades in the therapy of metastasizing malignancies by improving chemotherapeutic strategies and new approaches in radiation therapy. Genetic manipulation of tumor cells and of the tumor fighting immune system is hoped to add significant contributions to curative interventions in disseminated tumors. That we are still far from eradicating death by malignant growth is due ultimately to our limited understanding of the cascade of events resulting in metastasis formation, which until recently was believed to rely on multiple rounds of mutation and selection processes. This implies an individually specific history of each metastatic tumor, which would rule out uniform diagnostic and therapeutic concepts. When it was noted in a rat tumor model that the transfer of cDNA of a single gene, a CD44 variant isoform (CD44v) covering the exons v4–v7, sufficed to initiate metastasis formation of a locally growing tumor, hope was created that a "metastogene" may have been identified. Although the idea of CD44v expression as a unifying concept for tumor progression was not sustained, the discovery of CD44v-initiated metastatic spread allowed a conceptually new hypothesis on tumor progression as a consequence of the reactivation of genetic programs of ontogeny, stem cell differentiation, and/or lymphocyte activation. Since distinct CD44 isoforms play an important role in these processes, unraveling the functions of this family of molecules can indeed provide a cornerstone in the understanding of tumor progression. This article summarizes briefly the present knowledge on known functions of CD44 isoforms with particular focus on parallels between physiological programs and tumor progression.

Key words CD44 isoforms · Hematopoiesis · Lymphocyte activation · Signal transduction · Metastasis

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Abbreviations *CD44s* CD44 standard isoform · *CD44v* CD44 variant isoforms · *HA* Hyaluronate

Introduction

Tumor progression

There are excellent reviews in the field of metastasis formation [1–7], and the attempt to summarize or to add anything new is beyond the scope of this review. Let us recall intially the metastatic cascade: (a) loss of contact with the surrounding tumor cells or neighboring cells, as exemplified by the loss of E-cadherin [7, 8]; (b) breakthrough the basement membrane and penetration of vessel walls [6, 9, 10]; (c) survival of sharing forces in the blood stream [11]; (d) adhesion and penetration through vessel walls [4, 9, 10]; (e) expansion into foreign tissue [12–14]; (f) organization of supply with nutritients by vascularization [15–18]. It should be mentioned, finally, that the differentiation between hematogenous and lymphatic spread of tumor cells appears to be significant. While entering of the lymphatic system and the transport therein is less demanding than passing blood vessels and surviving the turbulence of the blood stream, "passive" metastasis formation by embolus formation within the capillary system and breaking through the walls after settlement of micrometastasis can occur only in hematogeneous metastasis formation. The observation that some tumors are known to metastasize exclusively either via the lymph or via the blood system reinforces the distinct requirements for each pathway [11].

Organ-specific metastasis formation

This metastatic cascade does not take into account the organ specificity of metastasis formation. However, from the clinical point of view this feature of tumor progression has long been common knowledge, and the "seed and soil" hypothesis was originally formulated over 100

years ago by Paget [19]. This postulates that tumor cells ("seeds") can grow only when they find the proper surrounding ("soil"). However, recent experimental findings support this view. Carcinoembryonic antigen is one of the candidates, which after more than 20 years, could be assigned functionally to the preferential homing of colorectal cancer cells into the liver [20]. The state of current knowledge has been comprehensively reviewed by Radinsky and Fidler [21], Fidler [22, 23], and other experts in the field [4, 13, 19, 24–34]. Interestingly, as with tumor progression in general, organ-specific homing and settlement also appear to be guided largely by adhesion molecules expressed in tissue-specific patterns by tumor cells and/or endothelial cells. In addition, growth factors produced, for example, by fibroblasts in a defined tissue context must also be considered.

Adhesion molecules and metastasis

Adhesion molecules are known to guide morphogenesis and organogenesis and are involved in the maintenance of organ structures [35–41]. Moreover, they are of the utmost importance for most functional activities of the immune system [42–49] and are thought to be involved in tumor progression [4, 12, 13, 30, 32, 50–56]. Their continously growing numbers are grouped into five families: integrins, selectins, cadherins, the Ig superfamily, and H-CAMs, the latter group including CD44 [42, 50, 57–65]. Especially integrins, the Ig superfamily, and H-CAMs include varying combinations of heterodimers by changes in glycosylation and in the protein structure, frequently due to alternative splicing of pre-mRNA [38, 42, 57, 66–70]. This complexity of structures is correlated with a multitude of functions. Cell adhesion molecules mediate either cell-cell or cell-matrix interactions or both. They are frequently also involved in signal transduction resulting in altered patterns of gene expression [8, 32, 36, 38, 42, 56, 57, 71–76]. As mentioned above, the metastatic cascade is fundamentally linked to repeated changes in tumor cell adhesiveness (recently reviewed in [4, 77]). It is therefore not surprising that CD44 is involved in tumor progression, but it is most surprising that variations in the expression of a single gene can create a family of proteins which display the whole array of possible functions of adhesion molecules in ontogeny, lymphocyte activation, and tumor progression.

The CD44 family of glycoproteins

CD44 comprises a family of glycoproteins with variable N- and O-linked glycosylation sites [78–86]. The so-called standard or hematopoietic form spans a region of seven extracellular exons, a transmembrane exon, and a cytoplasmic exon [87–89], which can be short (exon 9) or long (exon 10) [90]. Between exon 5 and exon 6 up to ten additional, so-called variant, exons can be inserted [68, 91–94]. Although multiple combinations of variant exons have been described, some are expressed differentially or are at least found predominantly on specific tissues such as the epithelial form [95], the keratinocyte type [96–97], or the reticulocyte type [98] (Fig. 1). Translation of the variant exons has been suggested to follow the 3′-5′ end rule. There are exceptions inasmuch as individual cells can express a multitude of combinations of splice variants, with the individual combinations not necessarily containing sequential exons. Finally, depending on the state of activation, individual cells can repeatedly change the splicing of CD44 pre-mRNA. The mechanisms which regulate alternative splicing of CD44 is unknown, but there are preliminary reports on regulation of the CD44 promoter by *ras* and of altered splicing after hyaluronidase treatment [99–100].

CD44 is an adhesion molecule with two binding domains for hyaluronate (HA) [101–107]. HA binding is influenced by the cytoplasmic tail [108–109] while the membrane proximal domain does not appear to be involved [110]. Not all CD44-positive cells bind to HA, but HA binding can be induced by cross-linking [111],

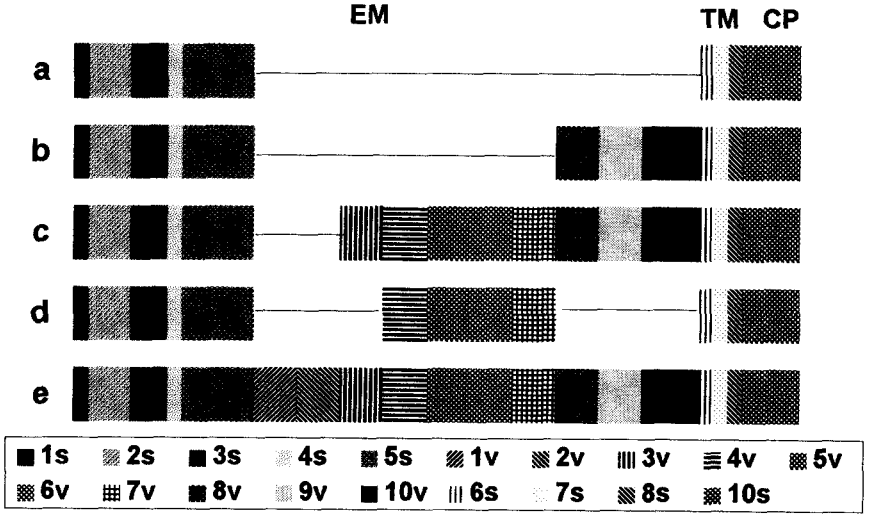

Fig. 1 Protein structure of CD44. *a*, The members of the family of CD44 glycoproteins are composed of a minimum of eight extracellular exons, one transmembrane spanning, and one short (exon 9) or long (exon 10) cytoplasmic exon. *e*, Between exon 5 and 6 up to ten variant exons can be inserted in multiple combinations. Some more frequent isoforms are named: keratinocyte type (exon v8–v10; *b*), epithelial type (exon v3–v10; *c*), meta (metastasis-associated)-1 (exon v4–v7; *d*)

which is thought to result either in conformational changes or in a redistribution of CD44 in the cell membrane [112]. Furthermore, O-glycosylation sites are important for the CD44-HA interaction [85]. CD44 also bind to fibronectin [113, 114], laminin and type IV collagen [115], and gycosaminoglycans [116]. The molecule is known to be involved in the assembly of the extracellular matrix [111, 117–118]. For some functions binding to the cytoskeleton via ankyrin is essential [85, 114, 119]. CD44 variant isoforms (CD44v), in particular, are linked via the ERM family to the actin based cytoskeleton [120]. There are three phosphorylation sites at the intracytoplasmatic tail, and binding to the cytoskeleton is not observed in the phosphorylated state [109].

CD44v are expressed less abundantly. Although most epithelia and the hematopoietic organs are CD44v-positive during ontogeny [121–122], expression of CD44v in the adult is restricted mainly to the skin, the epithelium of the gut, and a variety of glands [121, 123–125]. In all instances expression of CD44v is linked to a high rate of cell division [121–122]. It should be mentioned that even within the CD44v-positive tissues different cell layers express distinct CD44 variant isoforms. This suggests both a strictly regulated mechanism of splicing and divergent functions [67, 126–127].

CD44 and metastasis

The model

The possible involvement of CD44v in tumor progression was first described in a metastasizing rat tumor line [128]. This pancreatic adenocarcinoma predominantly expressed the variant exons v4–v7 [91]. Metastasis formation of the locally growing subline was initiated by the transfer of CD44v4–v7 [91], and metastatic spread was inhibited [129–130] by an antibody, 1.1ASML, recognizing an epitope on exon v6 [91, 131]. In the rat this phenomenon appears to be of general validity. In a variety of rat tumor lines with paired sublines either growing locally or metastasizing via the lymphatic system, expression of CD44v has been detected exclusively in the metastasizing sublines [132]. In line with this finding is the notion that transfection of different nonmetatasizing rat tumor lines with CD44v transfers the metastasizing phenotype. This is independent of the histology of the primary tumor and of the grade of dedifferentiation. The capacity to form lymph node metastases is correlated solely with the intensity of surface expression of CD44v (Hofmann et al., unpublished finding). Interestingly, in a rat colon carcinoma model it has also been noted that tumorigenicity is correlated with CD44v6 expression [133]. Experiments aimed at defining the important structural equivalent were able to exclude that any of the variant exons interfered negatively with metastatic progression (Sleeman et al., unpublished finding). Also, no interference of exons from the standard part of the molecule was observed (Kasuhiro et al., unpublished finding). Finally, transfection with exons v6 and v7 or with exon v6 as the only variant exon still confers metastatic behavior [134] (Kasuhiro, unpublished finding). However, it remains to be explored whether the variant exons by themselves, interactions between the standard exons and exon v6, or conformational changes by the insertion of variant exons are the structural equivalent to metastasis induction. Regardless of this, however, the observation that expression of CD44v initiates lymphatic spread of solid tumors in the rat has received much attention. It has been hypothesized that CD44v and in particular exon v6 may be of special importance in tumor progression (reviewed in [135–139]). Screening of human tumors did not unequivocally support this assumption (reviewed in [140]). However, for some human malignancies expression of CD44v is clearly correlated with tumor progression.

CD44 in human malignancies

Following the recognition that CD44v play an important role in the lymphatic spread of rat tumor cells, many institutions screened human tumors for the expression of CD44v and searched for correlations between expression profiles and prognostic paramters. Although data are not yet available for all types of malignancies, it is evident that expression of CD44v on metastatic tumors in the human is variable. In some tumors, such as neuroblastoma, there is no expression of CD44v [141], or tumor aggressiveness is even correlated with repression of CD44 expression [142]. Also, tumors arising from CD44v-positive tissues, especially the skin and squamous epithelium including the lung, appear to loose expression upon tumor progression [143–144] (Seiter et al., submitted). On the other hand, CD44v is frequently upregulated in tumors infiltrating the skin, but this appears to be associated with tissue injury rather than with tumor progression [145]. Other tumors such as prostate cancer [146] and gastrinomas [147] express, in contrast to their nonmalignant counterparts, CD44v even at early stages of malignant transformation and unlinked to metastatic progression. However, the progression of some tumors, including those in humans appears to be closely linked to CD44v expression. This has been described in breast carcinoma [148–150], bladder carcinoma [151], high grade non-Hodgkin lymphoma and large cell lymphoma [152–153], kidney carcinoma [154], high-grade glioblastoma and meningioma [155], and hepatocellular carcinoma [156]. Contradictory findings have been reported in some tumor systems. In colorectal carcinoma, for example, some groups have described a correlation between tumor progression and CD44v6 expression [100, 157–160] while others have detected no expression of CD44 splice variants or noted it early and independently of progression [161–163]. Upregulation of CD44v expression has been reported in cervical cancer by Dall et al. [164] but was not detected by another group (Woerner et al., submitted). Also in the case of gastric cancer evi-

dence supports correlation of CD44v expression with progression either only in the intestinal, less differentiated type [165–167] or generally [168–169]. The use of different sets of reagents may explain at least some of the discrepancies.

Interestingly, to the extent that a correlation between tumor progression and CD44v expression was noted, it was – unlike in the rat model – not essentially exon v6 that was upregulated in human malignancies. Instead, the expression of other variant exons has been described to be important for metastasis formation of human tumors, for example, exon v9 in kidney carcinoma [154], exon v10 in skin metastasis of melanoma (Seiter et al., submitted), exon v5 or exon v9 in some types of gastric cancer [165, 168], exon v5 for the settlement of melanoma cells in lymph node tissue (Seiter et al., submitted), exon v7-v8 in carcinoma of the cervix uteri [163], and exons v4 and v5 in hepatocellular carcinoma [156].

Although CD44v cannot be considered as a general metastasis marker in the human, for some types of malignancies a strong correlation between metastasis formation and CD44 expression has been demonstrated. Possibly of similar importance is the notion that some tumors explicitly downregulate expression of CD44. When one also considers that expression of CD44v initiates lymphatic spread of rat tumors, the CD44 family of glycoproteins appears an ideal model for examining the precise requirements of progressively growing tumor cells at each step in the metastatic cascade by defining the functional principles of distinct CD44 isoforms. Since tumor cells which gain in metastatic capacity recruit new and/or silence gene activities, it is also tempting to speculate that they adopt pathways of functional activities from those cells and organs in which activation or silencing of genes occurs physiologically [170–171]. This is frequently observed during ontogeny, in stem cell differentiation, and during lymphocyte activation.

Physiological and metastasis-associated functional activities of CD44

It has been proposed repeatedly that the multitude of CD44 isoforms corresponds to a multitude of functions. This view is based on the following observations: (a) expression of CD44 isoforms appears strictly regulated, (b) expression of CD44v is generally transient, (c) distinct cells express different CD44v, and (d) the same cell can express different CD44 isoforms depending on its state of activation. Although by no means are the functions of all possible CD44 isoforms known, some – in particular those of the CD44 standard isoform (CD44s) – are known, and there is initial evidence for activities of CD44v which allow the determination of whether tumor progression does indeed rely on the recruitment of physiological programs involving expression of CD44 isoforms.

Functional activities of CD44s and CD44v as substrate and cell adhesion molecules

CD44s is known as the principal receptor for hyaluronan, one of the major components of the extracellular matrix. By its unique structure as the longest molecule in the organism, hyaluronan is thought to be important particularly as a skid for cells. It has been shown that during development the expression of CD44s and that of hyaluronan coincides, and that, for example, in the limb bud the protruding edge is especially rich in hyaluronan and in brightly CD44-positive cells. Similar features account for the somite formation [172–173]. It has been suggested that hyaluronan/CD44 is involved in the formation of the early mesoderm, the differentiation of the craniofacial mesenchym, and the morphogenesis of the axial skeleton [174]. Interestingly, CD44 also degrades hyaluronan [175–178]. This function may be of importance in the formation of ducts, cavities, and canniculi as required in the formation of the respiratory tract, the homeostasis of cartilage tissue [177, 179], and the formation of dermal condensations [180]. Independently of the concomitant presence of hyaluronan, expression of CD44 has also been noted in instructive epithelia [172]. All of these findings are in accordance with the view that CD44 facilitates migration of cells on substrates of the extracellular matrix; they also suggest additional, as yet undefined activities.

A second function undoubtedly associated with CD44 is its involvement in lymphocyte "homing" (reviewed in [181]). There are several respects in which this is of physiological importance. One is the homing of mature lymphocytes in peripheral lymphoid tissues, in particular into lymph nodes. Lymphocytes bind to high endothelial venules via CD44 and binding can be inhibited by anti-CD44 antibodies [182–186]. This function is restricted to CD44s and is not mediated, for exampe, by the epithelial isoform of CD44 [187]. It is also known that CD44 is involved in the binding of bone marrow cells to stromal elements, where binding of myeloid cells in particular seems to function via HA binding [188]. Furthermore, mere seeding of stem cells on stroma layers requires CD44, but seeding can be inhibited by antibodies, which do not block HA binding (Khaldoyanidi et al., submitted). CD44 is involved in the binding of colony forming cells to fibronectin [189], in plasmocytoma cell–stroma interactions [190], in binding of lymphocytes to human umbilical vein endothelial cells [191], and in lymphocyte endothelial cell interactions in general [192]. It should be mentioned that lymphocyte binding is inducible [193], and in most instances, especially regarding HA binding, it is observed only after induction [111–112]. Evidence has recently been presented that migration of prothymocytes into the thymus is also guided by CD44, but not via HA binding [194]. CD44 also plays a role in the reappearance of T cells in the periphery after depletion protocols [195–196]. Experiments in the rat have revealed, in accordance with published evidence, that stem cell seeding, migration of prothymocytes, and homing of nonacti-

vated lymphocytes can be partially inhibited by anti-CD44s, but by neither anti-CD44v6 nor GST-CD44v fusion proteins covering the variant exons v4–v10 (M.Z., unpublished findings). Although further experiments are required for an unequivocal exclusion, all data available so far indicate that the homing and migration of both hematopoietic progenitor cells and mature lymphocytes into lymphoid organs is independent of the CD44v expression but is influenced by CD44s.

In addition to its function in lymphocyte homing into lymphoid organs, CD44 also is involved in the homing into nonlymphoid organs (reviewed in [197]) which is especially important in infectious and allergic and autoimmune reactions. CD44 is thought to be involved particularly in the extravasation of lymphocytes, but not in the migration process [198]. It has been described that T cell–keratinocyte binding is strengthened by anti-CD44 [199], that infiltration of B cells in the lacrimal gland depends on CD44 [200], and that T cell–astrocyte interactions are also CD44 mediated [201]. Furthermore, CD44 induces cell aggregation [202], which depends on its interaction with the cytoskeleton [203]. Also, upon lymphocyte–endothelial cell interaction syncapping of CD44 has been noted, which could play a critical role during recirculation and homing of activated lymphocytes in injured organs [204]. It has been described that CD44, by immobilizing macrophage inflammatory protein-1β, induces chemotaxis and adhesion of T cells to vascular cell adhesion molecule 1 in inflammatory processes. Finally, the binding of platelets to endothelial cells after tissue injury also appears to be mediated by CD44 [205].

In contrast to the homing of progenitors and naive lymphocytes in hematopoietic organs, there is evidence that CD44v are required for the homing of activated lymphocytes in nonhematopoietic tissues. The human skin abundantly expresses the so-called keratinocyte form of CD44, which contains the variant exons v3–v10 [96]. Although expression of exon v10 has been noted neither in the bone marrow nor during lymphocyte activation, lymphocytes infiltrating the skin strongly expressed exon v10, irrespective of whether malignantly transformed or in the course of infectious or allergic reactions. Concomitant expression of CD44v10 has also been noted on capillary walls in the surrounding tissue (Wagner et al., submitted). It is therefore tempting to speculate that expresssion of CD44v10 is fundamentally required, but also may be sufficient for infiltrating the tight connections of the epithelial layers of the skin. Interestingly, it has been reported that CD44v are linked to the ERM members of the cytoskeleton. The ERM family of molecules is closely related to the catenins, which are linked to cadherins, the major adhesive element of epithelial structures, which tight neighboring cell by homotypic binding. Whether CD44v10 also functions by homotypic binding, and whether CD44v10 represents a counterpart to E-cadherin remains to be explored. Furthermore, it will be interesting to evaluate whether the requirement of CD44v10 expression for homing into the skin represents a unique situation, or whether infiltration of nonlymphoid organs in general depend on expression of defined CD44v.

Linkage between metastasis formation and CD44-mediated migration and homing

A linkage between upregulation of CD44 expression and metastasis formation has been noted in a variety of tumors (reviewed in [140, 206–208]) particularly in hematopoietic malignancies (reviewed in [209]). In lymphoma and leukemia the level of CD44 expression is correlated rather with the dissemination than the degree of dedifferentiation (reviewed in [210]). This has been found in B cell acute lymphatic leukemia [211], multiple myeloma, where expression of CD44 correlates with homotypic adhesion [212], Burkitt's lymphoma [213], non-Hodgkin lymphoma [84, 214–216], and T lymphoma, where expression of CD44 coincides with increased tumorigenicity [217]. For a B cell hybridoma it has been described that expression of CD44s is accompanied by aggregation and metastasis formation [218].

High level of CD44s expression has also been noted on solid tumors, for example in melanomas [206, 219–221], where it is thought that CD44 plays a role in forming a leading lamella which is required for efficient locomotion, and that the chondroitin sulfate portion of CD44 is the critical component for the increased motility by interaction with type I collagen [222–223]. Similar notions have been described in gastric cancer [224], mesothelioma [225], breast carcinoma [226], glioblastoma, and meningioma [227–228]. In line with these findings is the observation that in ovarian tumors a decrease in tumorigenicity is apparently correlated with a decrease in CD44 [229]. Considering the underlying mechanism, it has been suggested that CD44 increases motility [224–226] or facilitates penetration by HA degradation or by interaction with the extracellular matrix [227]. The latter possibility is strongly supported by the view that melanoma metastasis formation is inhibited by a CD44-Ig fusion protein which inhibits binding to HA but not by mutated CD44-Ig fusion protein [230].

There are few reports which consider possible functions of CD44v in tumor cell migration and homing in tissue of foreign origin. In the rat model, where metastasis formation is transferred by transfection with CD44v4–v7 cDNA, we have excluded that CD44 variant exons facilitate either the migration or the embedding of tumor cells in the draining lymph node [132]. However, especially regarding the skin it appears that tissue-specific infiltration again may require and be accompanied by de novo CD44v expression. Jackson et al. [231] recently reported that a special variant isoform spans v3, v8–v10, or v8–v10, or v10 only. Exon v3 has been found to contain glycosaminoglycan-related sequences, which are known to act as reservoirs for growth factors in many tissues [232]. There is no ligand structure on the endothelial cells. However, the authors suggested that cytokine

production may be initiated via CD44v3, particularly by keratinocytes, monocytes, and dendritic cells in the skin [233]. Of special interest also appears to be the observation that basal and spindle cell carcinoma, which do not or only rarely metastasize, express the skin-associated pattern of CD44v at early stages of tumor growth but loose expression of exon v10 when leaving the epidermal tissue. The same accounts for squamous cell carcinoma of the head and neck region, where CD44v8–v10 is most strongly downregulated on metastatic tissue (Seiter et al., submitted). Melanomas, on the other hand, strongly express CD44v10 when infiltrating the skin but loose this particular variant isoform when metastasizing to the draining lymph node or upon in vitro culture (Seiter et al., submitted). Finally, intracutaneous lymphoma expresses CD44v10, which is not expressed on hematopoietic precursor cells or during systemic activation of lymphocytes. It is, however, found on intracutaneous lymphocytes both during allergic reactions and during inflammation of the skin (Wagner et al., submitted). These features of shared expression of CD44v10 on activated and malignant lymphocytes related to the homing organ suggests joint features in organ-specific metastasis formation and lymphocyte infiltration in autoimmunity or in response to injury. Taken together, the data are strongly suggestive that special variant isoforms facilitate organ-specific homing of both lymphocytes and tumor cells.

CD44 in hematopoiesis and hematopoietic malignancies

CD44 is known to play important roles in the differentiation and proliferation of hematopoietic progenitor cells in the bone marrow microenvironment [234]. As early as 1990 Miyake et al. [235] described that in long-term bone marrow cultures of the mouse no cobblestone areas appear in the presence of anti-CD44s, and that nonadherent progenitors do not develop. CD44 have been shown to be necessary for both myelopoiesis and for lymphopoiesis [235–238] (Khaldoyanidi et al., submitted). Inhibition of hematopoiesis by anti-CD44s is restricted mainly to stem cells and early progenitors, i.e., anti-CD44s display no [235] or minor effects (Khaldoyanidi et al., submitted) on the colony formation of committed progenitors in soft agar cultures. Since anti-CD44s interfere predominantly with the maturation/expansion of stem cells and/or early progenitor cells, one can presume that expression of CD44s is required either for interactions between stem cells/progenitor cells and stromal elements, or that by ligand binding growth-promoting signals are transfered into the hematopoietic stem cell/precursor cell. The two possibilites are not mutually exclusive. In fact, at least part of the anti-CD44s mediated blockade relies on the inhibition of cell division. When freshly harvested bone marrow cells were incubated with IM-7 and then treated with [^{3}H]thymidine, a significant decrease was noted in the number of stem cells which had undergone suicide upon transfer into lethally irradiated mice (Khaldoyanidi et al., submitted).

Establishment of rat LTBMC in the presence of anti-CD44v6 revealed that maturation particularly of the adherent stem cell population requires expression of CD44v. As in mouse LTBMC, anti-CD44s prohibit the development of nonadherent progenitors at least during the starting 5–7 weeks of culture. Anti-CD44v6 displays only a minor effect during the first 2–3 weeks. Thereafter the cultures contained exclusively stromal cells, and hematopoiesis did not recover after omission of anti-CD44v6, while it did recover in cultures containing transiently anti-CD44s (M.Z., unpublished finding). Since maturation of hematopoietic stem cells requires basical interaction with the stromal environment (reviewed in [239]) the question arises of whether CD44v6 expression is required for stroma formation, and/or whether CD44v6 is involved in stem cell–stroma interactions. In fact, stroma formation of rat bone marrow is significantly delayed in the presence of anti-CD44v6. Considering that stromal cells do express CD44v6, it is tempting to speculate that by CD44v6 (stem cells)-ligand (stroma cells) interaction signals are transduced, which facilitates stroma formation. Since, on the other hand, stem cell maturation is also completely inhibited in the presence of anti-CD44v6, a binary mode of signal transduction must be assumed.

Although CD44 may possibly be replaced by distinct adhesion molecules, CD44 apparently plays an essential role in stem cell proliferation, expansion, and maturation. According to published evidence and in line with our findings, the CD44 standard isoform provides (upon ligand interaction) proliferation initiating signals for early progenitors of all three hematopoietic lineages. CD44v, on the other hand, may be involved primarily in transducing signals between stromal cells and stem cells, which initiate differentiation.

Among the joint functions of CD44 isoforms in lymphocyte maturation and tumor progression, malignancies of the hematopoietic system should be considered in particular since these tumors frequently resemble early stages of development. Indeed, as outlined above, many hematological malignancies are accompanied by high expression of CD44, and progressive states are defined by upregulation of CD44s and CD44v6. So far, however, a possible growth-inhibiting potential of anti-CD44 has not been evaluated with native leukemia, lymphoma. However, it has been shown that a CD44s-negative Burkitt's lymphoma line (Namalwa) transfected with CD44s-cDNA displays increased tumorigenicity and metastatic potential upon intravenous injection [240]. Furthermore, tumor growth can be inhibited by a CD44s-Ig fusion protein [241]. Interestingly, a slightly reverse effect has been noted with CD44v-transfected Namalwa cells. A possible explanation is that CD44s confers growth-promoting activities while CD44v expression is prone to differentiation. So far this view is merely speculative; however, it is possible that it could be experimentally verified.

Costimulatory function of CD44 in lymphocyte activation and tumor cell expansion

There is ample evidence that CD44, as most adhesion molecules, functions as costimulator in T cell activation [242–249]. Interestingly, some antibodies are stimulatory together with anti-CD2 but not with anti-CD3. The latter have been found to stimulate palmitoylation of CD44 [199]. Cross-linking via anti-CD44 also leads to activation of cytolytic T cells and is a trigger for natural killer cells, the pathway of activation strongly resembling that for activation via the T cell receptor [250–252]. It also is known that the association with the cytoskeleton is especially important for T cell activation via cross-linking of CD44 [253]. Under more physiological conditions of antigen-specific activation CD44 has been shown to be involved in enhanced binding of dendritic cells to T cells [202] and to trigger the chondroitin sulfate form of the invariant chain to function as a costimulus [254]. CD44 promotes homotypic adhesion via lymphocyte function associated antigen 1 [255], and by HA binding interleukin-2 production and release of trypsinlike esterase by cytotoxic T lymphocytes may be triggered in a PTK-dependent fashion [252, 256–257].

Thus there is no question of the functional importance of CD44 as costimulatory molecule in T and probably also B cell activation. However, few of these studies differentiated between CD44s- and CD44v-mediated effects. We have begun to solve this question focusing on T cell responses in the rat, because rat T cells upon antigenic or mitogenic stimulation are known to express only one or two (CD44v6 or CD44v6–v7) of the ten variant exons. Our blocking studies with anti-CD44s and anti-CD44v6 have clearly shown that functional activity of CD44 in the rat during the activation process is independent of the upregulation of CD44s but is mediated by CD44v6 (CD44v6–v7). The conclusion is based on the following observations: (a) T cell dependent and T cell independent immune responses both in vivo [258] and in vitro (Arch, unpublished finding) are significantly inhibited in the presence of anti-CD44v6 but are not altered by anti-CD44s; (b) proliferation and cytotoxicity assays set up under limiting dilution conditions reveal that the frequency of responding cells is reduced by anti-CD44v6; (c) when purified T cells are cultured on anti-T cell receptor coated plates, a strong costimulatory effect of anti-CD44v6 is observed. These data are interpreted in the sense that CD44v is required for the activation process itself but, in distinction to CD44s activity in the mouse, not for effector functions (M.Z., unpublished finding). Since both ligand binding and cross-linking of CD44v6 at the cell surface initiates signals leading to lymphocyte proliferation and maturation, two possible modes of CD44v6 function should be considered: (a) Signals are transduced into the antigen-presenting cell, which becomes activated. This could result in increased cytokine production, as described for IL-1β, TNF-α, TNF-β, insulin-like growth factor-1, macrophage colony-stimulating factor [259–262] and interleukin-2 [263–264] by activated CD44 or in an augmentation of presentation as seen after binding of CD44 to the chondroitin sulfate form of the invariant chain [254]. The observation that lymphocyte activation is severely impaired after preincubation of antigen presenting cells with anti-CD44v6 supports this view. (b) Alternatively, but not mutually exclusive by cross-linking or ligand binding of CD44v6, signals are transfered in the T or B lymphocyte which initiate proliferation or activation of genes associated with immune response. The latter view is supported by the observation that upon cross-linking of CD3 anti-CD44v6 supports T cell proliferation. It thus appears that in the rat predominantly CD44v (v6 or v6–v7) are involved in the process of T cell activation, and that CD44v6/CD44v6–v7 fulfills divergent functions, i.e., modulates the activity of both antigen-presenting cells and the lymphocyte. Experiments are in progress to clarify the underlying molecular events.

Do tumor cells which have settled in the draining lymph node require CD44v6 in a similar way? Preliminary evidence suggests that this is in fact the case. An increased proliferation rate is noted upon culturing of tumor cells on anti-CD44v6 coated plates. This is interpreted to indicate that cross-linking of CD44v6 on the tumor cell initiates signals with growth-promoting activity, as has been observed upon cross-linking of the TCR concomitantly with CD44v6 on lymphocytes. Furthermore, CD44v-positive tumor cells preferentially adhere to dendritic cells, and the proliferation rate of tumor cells is clearly augmented in the presence of antigen-presenting cells. Adhesion of tumor cells can be blocked by anti-CD44v6, and as a consequence the growth advantage for tumor cells supplied by antigen presenting cells is abolished. Finally, there is preliminary evidence that cytokines secretion is augmented upon CD44v-ligand binding, for example, upregulation of tumor necrosis factor and interleukin-1 secretion by CD44-mediated monocyte–tumor cell interactions has been described [259, 265]. It thus appears that via CD44v on both tumor cells and lymphocytes signals can be transduced in the CD44v-positive and in the CD44v ligand-bearing cell, which in addition to proliferation also initiates cytokine production.

CD44 isoforms are so far the only molecules which can confer metastatic capacity to nonmetastasizing tumor cells. In view of this it could be of great value to elucidate the molecular mechanisms underlying the various physiological functions of CD44 isoforms, and thereby one (as *pars pro toto*) program of tumor progression. The physiological patterns of expression of CD44 suggest that distinct exons/isoforms of the molecule are involved in cell-cell and cell-matrix adhesion as well as in cell motility. Ligand binding may be required for, or at least may facilitate, both organ-specific homing and signal transduction. There is evidence that the latter initiates cell proliferation, differentiation, and/or cytokine production as well as activation of proteases and other enzymes. This contribution attempts to elucidate whether and when in the metastatic cascade tumor cells make use

of "physiological functions" of CD44 isoforms. There is convincing experimental evidence that CD44 in tumor progression shares many features with CD44 activities in developmental programs, stem cell differentiation, and lymphocyte activation. However, since the coordination of distinct functions to defined CD44 isoforms is far from complete, many questions remain unanswered. The molecular mechanisms underlying the distinct functions of CD44 isoforms as a model system of tumor progression are yet to be unraveled (Fig. 2).

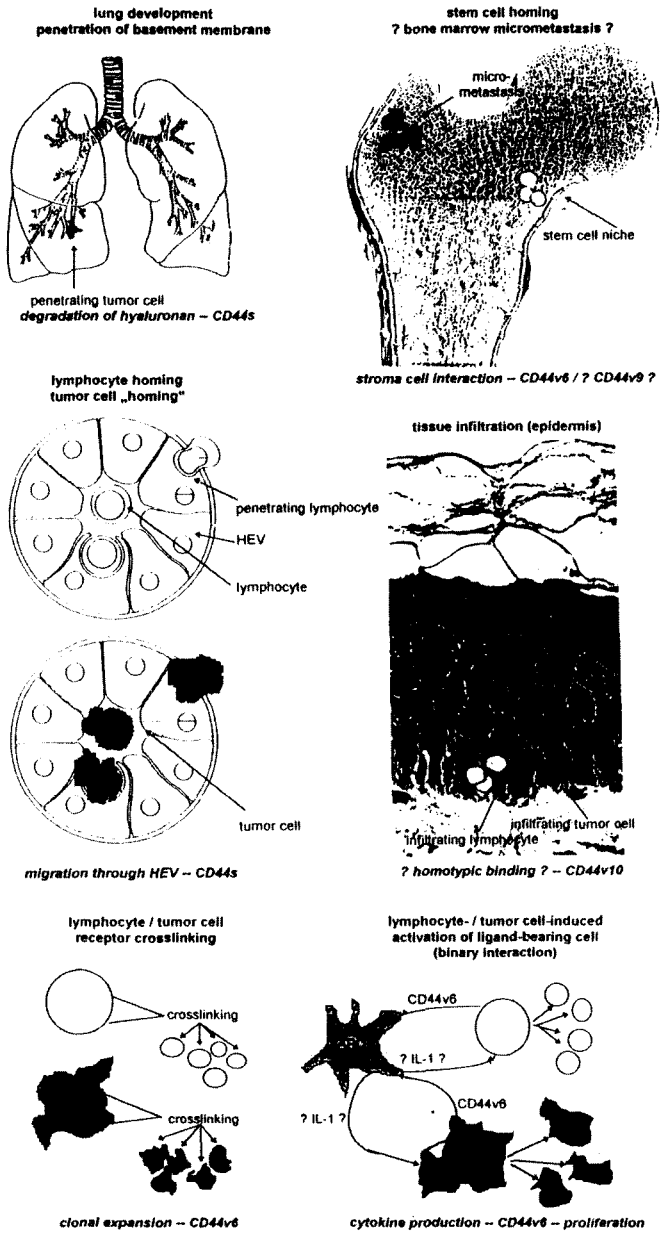

Fig. 2 Functional activities of CD44. Examples of analogous physiological and metastasis-associated functions of CD44: degradation of hyaluronan/penetration of basement membrane; settlement of stem cells/"isolated tumor cells" in the bone marrow microenvironment; lymphocyte/tumor cell motility; induction of cytokine production, for example, in antigen-presenting cells by lymphocyte/tumor cell receptor engagement; growth promotion in lymphocytes/tumor cells by receptor cross-linking; immigration into nonhematopoietic tissue during immune responses, autoimmune reactions, organ-specific metastasis formation. Evidence for the involvement of defined exons is circumstantial in most instances; *parentheses*, hypothetical analogies

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