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CD44 in hematological neoplasias

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Abstract

The CD44 protein family spans a large group of transmembrane glycoproteins acquired by alternative splicing and post-translational modifications. The great heterogeneity in molecular structure is reflected in its various important functions: CD44 mediates (i) interaction between cell and extracellular matrix (ECM), (ii) signal submission e.g. by acting as co-receptor for membrane-spanning receptor tyrosine kinases or by association with intracellular molecules initiating several signaling pathways, and (iii) anchor function connecting to the cytoskeleton via the ezrin-radixin-moesin (ERM) protein family. The expression pattern of the different CD44 isoforms display strong variations dependent on cell type, state of activation, and differentiation stage. In hematopoietic cells CD44 mediates interaction of progenitor cells and bone marrow stroma during hematopoiesis, regulates maturation and activation induced cell death (AICD) in T cells, influences neutrophil and macrophage migration as well as cytokine production, and participates in lymphocyte extravasation and migration. CD44 is involved in development and progress of hematological neoplasias by enhancement of apoptotic resistance, invasiveness, as well as regulation of bone marrow (BM) homing, and mobilization of leukemia initiating cells (LIC) into the peripheral blood. Thereby altered CD44 expression functions as marker for worse prognosis in most hematological malignancies. Additionally CD44 expression levels can be used to distinguish between different hematological neoplasias and subtypes. Concerning new treatment strategies CD44 displays promising potential either by direct targeting of CD44 expressed on the malignant cells or reversing an acquired resistance to primary treatment mediated through altered CD44 expression. Former can be achieved by antibody or hyaluronan (HA) based immunotherapy.

Keywords: CD44, hematological malignancies, CD44 antibody, hyaluronan

Introduction

The class I transmembrane glycoprotein CD44 has first been described in 1980 as a surface molecule on T-lymphocytes, cortical thymocytes, and granulocytes [1]. It plays an important role in cell proliferation, migration, survival, and apoptosis. Due to its various functions in physiological as well as pathological processes, CD44 has been given several names as for example phagocytic glycoprotein 1 (Pgp-1), Hermes antigen, and extracellular matrix receptor type III (ECM-III) [2]. In this review we convey structural understanding of the CD44 protein and outline the physiological function on molecular and cellular level in order to provide a broad background for comprehension of CD44 engagement in hematological neoplasias and potential strategies for diagnosis and therapy of these malignancies.

Genomic organization and splice pattern

The highly conserved gene of the CD44 transmembrane protein family is located on the short arm of chromosome 11p13 in humans and on chromosome 2 in mice. It spans approximately 50 to 60 kb of genomic DNA and codes for about 360 amino acids in 19 exons in humans, and 20 exons in mice. It comprises two kinds of exons, constant and variable ones. Former encode the extracellular globular part (exon 1-5), a short stem as connection to the cell membrane (exon 16 and 17) and the transmembrane domain (exon 18). Exon 19 and 20 are subject to alternative splicing creating either a short or more often a long cytoplasmic tail [3]. The exons 6 to 15 are variable (v1-10), enlarging the stem on its distal site and forming several distinct CD44 isoforms, referred to as CD44 variants (CD44v1-10) (Fig. 1). Different combinations of the variable exons create a CD44 repertoire of several dozen isoforms. CD44 is a mainly acidic charged molecule with a $t_{1/2}$ turnover of approximately 8 hours [rev. by [2, 4, 5].

The splicing process can be influenced by several factors including cytokine and growth factor (GF) stimulation: Without detectable changes in total CD44 m-RNA levels an upregulation of CD44v3, v5, v6, v7, v8 and v9 is obtained by treatment with 12-O-tetradecanoyl phorbol-13-acetate (TPA), insulin-like growth factor-1 (IGF-1), and platelet-derived growth factor (PDGF) [6]. IL-1 upregulates CD44 standard and induces appearance of v3- and v6-containing splice variants, most likely mediated by Egr-1 [7]. Further Ras [8], OPN [9], and poly(rC)-binding protein 1 (PCBP1) involvement [10], as well as DNA damage result in a changed splicing pattern of CD44 [11].

Protein structure

The CD44 protein core has a molecular weight of only 37 kDa but post-translational modifications and insertion of variable exons enlarge it up to 200 kDa. Lacking all variable exons, CD44 standard (CD44s) is the smallest isoforms with a protein product size of approximately 80 kDa. Six cysteine residues creating disulfide chains form the globular shape of the distal extracellular region that serves as a docking site for several elements of the extracellular matrix (ECM). The stem contains possible proteolytic cleavage sites on its carboxyl terminal end. The whole extracellular domain is subject to post-translational modifications: N-linked glycosylations, mostly seen in the globular and variable region, and O-linked glycosylations as well as the attachment of glycosaminoglycans usually found in the stem. The hydrophobic transmembrane domain embodies a cysteine residue that appears to play a role in CD44 oligomerization. The cytoplasmic tail embodies 6 putative serine phosphorylation sites and provides a connection to the cytoskeleton and to the tyrosine kinase family [rev. by [2, 4, 5, 12, 13]. Recent experiments assume that the juxtamembrane region of the cytoplasmic tail runs parallel to the negatively charged inner membrane because of its mostly basic residues. Contrarily the distal part, containing basically acidic residues, would project into the cytoplasm (Fig. 2) [14].

CD44 exists membrane bound and soluble. The latter can be generated by proteolytic cleavage of e.g. membrane-type 1 matrix metalloproteinase (MT1-MMP) [15]. This shedding of CD44 from the membrane can be triggered e.g. by oncostatin M (OSM), transforming growth factor beta 1 (TGF-beta 1) [16], and EGF [17].

Expression pattern

CD44s is almost ubiquitously expressed whereas physiological CD44v expression is restricted to a subset of tissues. Despite CD44s expression on e.g. connective tissues, blood vessels, and muscle [18], highest expression is observed on hematopoietic cells. Therefore CD44s is also referred to as hematopoietic CD44 (CD44H) [12]. Basically epithelial cells express CD44 variants, CD44v9 being the dominant isoform. It is expressed in most stratified squamous epithelia showing particular high expression levels in oesophagus, skin, and tonsil. Additionally CD44v6 and CD44v4 are expressed in epithelial cells, although in smaller amounts. The longest isoform containing all variable exons is expressed in keratinocytes. Within the individual tissues generative cells display the highest amount of CD44v [13, 18].

CD44v expression is transiently upregulated on activated T cells and cells of the innate immune system [12]. The expression pattern of the different variants of CD44 varies during lineage commitment, e.g. CD44v6 is upregulated in monopoiesis and downregulated in granulopoiesis [19].

CD44v6 is found to confer metastatic behavior to non-metastatic tumor cells [20] and plays an important role in various tumor entities concerning pathophysiology and prognosis. As reviewed by Heider et al. a high frequency of positive cells up to hundred percent is found in squamous cell carcinomas of head and neck, oesophagus, lung, skin, and cervix as well as in metastases of these tumors. In adenocarcinoma, high levels of CD44v6 are observed in breast cancer cells and even higher levels in cells of metastatic lesions. In cells of Barrett's, lung, gastric, pancreatic, colon, endometrium, and prostatic cancer, more varying levels are reported, displaying a more moderate expression rate. Also in thyroid carcinoma and basal cell carcinoma CD44v6 is frequently expressed [21].

Additionally CD44 has been proposed to identify tumor initiating cells, also referred to as cancer stem cells, in a multitude of different tumor entities as e.g. breast cancer [e.g. [22], colon cancer [e.g. [23], pancreatic cancer [e.g. [24], and liver cancer [e.g. [25] and thereby plays an important role in tumor pathogenesis, prognosis and therapy strategies.

Ligands and protein interactions

Protein interactions of CD44 are divided into three groups according to the compartment the interaction takes place. Extracellular, CD44 mediates outside-in signaling and the connection to ECM and surrounding cells. The cytoplasmic tail initiates intracellular signaling cascades and creates the connection to the cytoskeleton, thereby influencing cell shape and migration. The membrane region is characterized by recruitment of proteins to the membrane and interaction with membrane spanning receptors.

Extracellular region: CD44 binds to several components of the ECM: fibronectin, collagen type I, and type IV, laminin, osteopontin (OPN), and hyaluronan (HA), its principal ligand. Thereby HA fulfills a passive 'linking' function between CD44 expressing cells as well as signal transduction [2, 13]. The binding activity of HA to CD44 in general displays a great variability dependent on CD44 surface expression and clustering [26], insertion of variant exons in the CD44 molecule [6], and post-translational modifications of CD44 [among others [27, 28] and can be regulated by cytokines.

CD44/HA signaling itself can be affected by the molecular weight of HA e.g. during inflammation: While low molecular weight HA (LMW-HA) stimulates cell growth, high molecular weight HA (HMW-HA) inhibits proliferation [29]. Furthermore, the localization of the CD44/HA interaction at the cell surface has influence on the mitotic spindle axis formation. Here, binding of HA to CD44 at the apical membrane results in parallel orientation of the spindle, while ligation at the basal membrane leads to increased spindle axis rotation [30].

In hematopoietic stem cells (HSC), even though there is a high CD44 expression on CD34+ hematopoietic cells, spontaneous binding activity to HA is low. Binding affinity can be induced by stem cell factor (SCF), GM-CSF, and IL-3 without affecting the amount of total CD44 expressed on the cell surface. This effect might be due to conformational changes of CD44. Thereby pre-existing 'inactive' CD44 with low HA binding affinity would be activated by interaction of cytoplasmic tail and cytoskeleton [31]. Also monocytic CD44 can be transferred into a high (e.g. by TNF-alpha) or low (e.g. by IL-4) HA-affinity state by cytokines via post-translational modifications as N-linked glycosylation or chondroitin sulfate modification [32, 33]. Similarly T cell binding to HA is regulated by cytokines (e.g. IL-2, IL-8, TNF) activating CD44 [rev. [27]. Additionally antigen-activation of T cells enhances the affinity of their surface CD44 to HA [34].

CD44 plays a pivotal role in cell-cell interaction by interaction with integrins. Cooperation of CD44 with alpha4beta1 integrin, also referred to as very late antigen-4 (VLA-4) mediates adhesion of hematological progenitor cells to the bone marrow [35] and plays an important role in the process of leukocyte extravasation [36]. CD44v ligation by OPN induces integrin activation via Src-signaling leading to increased survival of cells [37]. Furthermore, cross-linking of CD44 results in higher expression levels of lymphocyte function-associated antigen-1 (LFA-1) and VLA-4 which are responsible for enhanced transendothelial migration of tumor cells [38].

Transmembrane region: Additionally to transmembrane proteins and components of the ECM, several soluble molecules are reported to bind to CD44 as e.g. members of the galectin family and several GFs (see table 1 and 2) and trigger intracellular signaling cascades. Hereby CD44 recruits GFs to the cell membrane and mediates GF interactions with their respective receptor tyrosine kinase. By this means CD44 functions as co-receptor for a wide range of receptor tyrosine kinases as members of the ErbB family, c-Met, VEGFR-2, and BMP receptor type II (see table 3) [rev. [13]. Further CD44 signaling activates transcription and translocation of MMPs to the cell surface. Thereby CD44 enhances invasiveness of tumor cells either by anchoring or increased secretion of proteolytic active MMPs (see table 4) [39-41].

The cytoplasmic tail cooperates with intracellular molecules as members of the Src tyrosine kinase family including c-Src, Lyn, Fyn, and Lck [42-45], Moesin-Ezrin-Radixin-Like Protein Merlin [46], T-cell lymphoma invasion and metastasis-inducing protein 1 and 2 (Tiam1/2) [47], intracellular OPN [48], as well as members of the Smad protein family [14, 49, 50]. Hereby c-Src signaling stimulates microRNA-mediated down-regulation of tumor suppressor gene transcription [51].

Intracellular region: The connection to the cytoskeleton is provided by ankyrin and the ezrin-radixin-moesin (ERM) protein family functioning as cross-linkers to the actin filaments. The ERM proteins have a well conserved FERM domain in common that binds to CD44, and possess a carboxyl terminal tail linking to F-actin [52, 46]. Affinity is regulated by phosphorylation of either the ERM protein family or the cytoplasmic tail of CD44, latter mediated by protein kinase C (PKC). Likewise ankyrin activity which mediates contact to the cytoskeleton component spectrin depends on its phosphorylation status. Thereby it modulates HA-dependent cell adhesion and motility. [13, 14]. Ankyrin binding is further modulated by RhoA and Rac1, members of the Rho-GTPase family [53]. Activation of both, RhoA and Rac1, is mediated by CD44 itself: CD44/MMP-9 activates TGF-beta [54] which is shown to regulate RhoA activity, rearrangement of the cytoskeleton, and adhesion [55]. Further Rac1 activation proceeds via HA-ligation to CD44, leading to actin cytoskeleton and cell reorientation [56]. Besides direct interactions, CD44 influences filament organization by co-activation of several tyrosine kinases resulting in downstream phosphorylation of cytoskeleton proteins [43] and F-actin rearrangement [44].

Intracellular signaling cascades

Complex intracellular signaling cascades, including the PI3K/Akt and the Ras/ERK pathway, mediate CD44-regulated adhesion, migration, proliferation, survival, apoptosis, and differentiation via three distinct mechanisms:

(i) Activation and association of intracellular molecules to the cytoplasmic tail of CD44 and downstream phosphorylation.

Hereby e.g. complex formation with Lyn leads to subsequent regulation of Akt phosphorylation, modulation of the actin-binding protein cofilin, and enhanced migration as observed in colon carcinoma [61]. Further CD44/HA-activated Rho-kinase increases cell motility by phosphorylation of myosin phosphatase and consecutive cytoskeletal activation, and confers ECM degrading properties by triggering MMP-9 and MMP-2 secretion. Additionally PI3K signaling is induced and increases cell proliferation and survival [39]. The ERK1/2 pathway can be stimulated e.g. by CD44/CD74 complex activation, which leads in turn to Scr association with the cytoplasmic tail of CD44 and subsequent ERK1/2 phosphorylation [66]. Furthermore association to the ERM protein family mediates not only cell motility as described above but also cell death and survival as seen in Jurkat cells where Fas-mediated apoptosis can be modulated by CD44s induced downstream ezrin/actin interactions [63].

(ii) Cleavage and subsequently translocation of the cytoplasmic tail of CD44 into the nucleus followed by transcription of several target genes inclusive the CD44 gene itself [57].

(iii) Downstream signaling of the CD44-activated receptor tyrosine kinases.

The epidermal growth factor receptor (EGF-R) for instance can be activated by CD44 co-localization and trigger the Akt as well as the ERK pathway. HMW-HA induced CD44/EGF-R colocalization results in protein kinase C (PKC) phosphorylation by activated EGF-R, Akt and Rac-1 activation, focal adhesion kinase (FAK)-mediated upregulation of MMP-2 secretion, and enhanced cell motility [59]. Further TGF-beta1 stimulated CD44/EGF-R complex formation is followed by ERK1/2 signaling and fibroblast differentiation [69].

Physiological function in cells of hematopoietic origin

CD44 is involved in a wide range of important and very different functions: Cell proliferation, wound healing, angiogenesis, migration, homing, hematopoiesis, differentiation, immune response, and cell survival. Many of these functions are regulated by CD44-mediated cytokine production and secretion (table 5) and have been reviewed extensively [among others [2, 13, 70]]. Here we focus on the role of CD44 during hematopoiesis and in mature blood cells.

CD44 plays two pivotal roles in early hematopoiesis: (i) mediation of the interaction of the progenitor cells with their respective niche in the bone marrow (BM), (ii) stimulation of cell proliferation and differentiation by regulation of local cytokine secretion [31, 35, 71, 72].

During fetal erythropoiesis CD44 expression on erythroid progenitor cells as well as on fetal hepatoblasts declines during maturation in order to prepare cells to leave the liver, suggesting a hematopoiesis dependent expression of CD44 [73]. Likewise in adult erythropoiesis, decreasing CD44 expression is found. Therefore the authors claim CD44 to be a better marker for discrimination between erythroblasts at different stages of development than CD71 [74].

Similarly during B cell maturation in the bone marrow, CD44 displays a very organized expression pattern characterized by two waves of downregulation: After expression of CD44 on uncommitted CD34 positive progenitor cells, there is a loss of CD44 at the very early stage of B cell development. Thereafter CD44 expression increases, followed by a second wave of down-regulation before B cells regain CD44 when entering the periphery [75]. A possible reason for this expression pattern might be the proliferation regulating effect of CD44. CD44 expression occurs at the same time as RAG-1 expression and thereby immunoglobulin chain rearrangement is down-regulated, and proliferation is increased. During subsequent immunoglobulin light chain recombination CD44 expression decreases again.

During T cell maturation CD44 mediates homing of progenitor cells from the BM to the thymus where T cell differentiation and selection takes place. Furthermore intrathymic expansion of immature T cells through the different zones is regulated by CD44v6. Hereby CD44v6 cross-linking promotes proliferation and reduces apoptosis in early thymocytes [76]. Clustering of CD44 and Lck leads to F-actin rearrangement via PI3K activation and regulates spreading of mature T cells [44].

Regulation of apoptosis plays an important role in mature T cells, especially in context of activation induced cell death (AICD). CD44 reduces Fas-mediated apoptosis in T helper 1 cells (Th1 cells) and increases effector cell survival. Thereby CD44 contributes to memory Th1 cell generation [77]. At the same time CD44 is able to promote AICD in T cells by at least two distinct mechanisms: (i) up-regulation of Fas ligand surface expression on peripheral T cells [78], (ii) Fas independent induction of apoptosis in activated T cells via HA [79].

Extravasation of mature lymphocytes depends on at least three different CD44/protein interactions: (i) CD44 mediates lymphocyte rolling and adhesion to endothelial HA. Affinity is regulated by conformational shifts of latter. This results in low and high binding activity of cells to HA. Hereby the change between these two states is essential for unimpaired rolling [80]. (ii) Binding of CD44v to different members of the selectin family mediates rolling via selectin-dependent tethering [81]. (iii) Thus firm adhesion, essential for subsequent squeezing through the endothelial cells, leads to CD44/VLA-4-mediated transendothelial migration [36].

In macrophages CD44 regulates migration behavior and cytokine production. CD44^{-/-} macrophages show decreased response to chemoattractants, implied by impaired migration [82]. Ligation of CD44v6 and v7 on monocytes trigger the release of GM-CSF and IL-6, which thus leads to increased proliferation of myeloid and lymphoid progenitor cells [72]. In cooperation with OPN, CD44 inhibits the expression of IL-10 an anti-inflammatory cytokine, promoting a cytotoxic immune response [83]. In cooperation with HA, CD44 displays protective effect against septic response [84]. On the other hand, CD44v7 ligation in mononuclear cells in an inflammatory setting is accompanied with apoptosis induction [85]. Taken together these findings indicate a crucial role of CD44 not only in stimulating the immune response but also in limiting an excessive reaction. Expression of macrophage inflammatory protein (MIP)-1alpha, MIP-1beta, cytokine responsive gene-2 (CXCL-10), monocyte chemoattractant protein-1 (MCP-1), as well as IL-8 can be downregulated by blocking of CD44 by antibodies in macrophages [86].

Further cell polarization, migration, and migration speed in neutrophils are dependent on CD44. CD44^{-/-} neutrophils display impaired migration concerning direction and speed and

reduced activation of RhoA [87]. Additionally CD44 ligation triggers neutrophil apoptosis in vitro [88]. Similarly Langerhans and dendritic cell migration is mediated at least in parts by CD44, since antibodies against CD44 block OPN-induced cell migration to lymphatic organs in mice [89]. Also the proliferation of mast cells is regulated by CD44 [90].

Hematological Neoplasias

Acute lymphoblastic leukemia

Being the most common malignancy in childhood, acute lymphoblastic leukemia (ALL) displays very young patient pattern and requires well-considered treatment strategies to minimize treatment related morbidity, mortality, and disease relapse. Total CD44 as well as CD44v6 have been identified as prognostic marker, and particular high or low CD44 expression patterns have been reported for several cytogenetic or prognostic subgroups in ALL.

In a small cohort of 16 pediatric patients CD44v6 expression was observed mainly in the medium and high risk group indicating a possible association between CD44v6 expression and unfavorable outcome [91]. This is consistent with the finding that high CD44v6 mRNA levels correlate with increased risk of relapse (n=21). Further expression of CD44v6 in ALL cell lines was associated with accelerated engraftment in NOD/SCID mouse transplantation experiments [92]. Contrarily, in a group of 97 pediatric B precursor ALL patients with neither known adverse nor favorable cytogenetic features no association between CD44v6 expression and prognosis was seen. On the other hand, total CD44 surface expression was identified as an independent predictor of disease relapse in the latter cohort [93].

In pediatric ALL, CD44 and CD27 surface expression patterns have been described for different ALL subtypes and prognostic groups. CD44 surface expression in diagnostic bone marrow samples measured by flow cytometry displayed (i) concordance between high CD44 expression and high risk T-ALL, (ii) significant lower CD44 expression in the TEL/AML1 ALL subtype together with high CD27 expression, and (iii) double CD44/CD27 expression pattern typically seen in bcr/abl positive subtypes [94].

Acute myeloid leukemia

CD44 signaling plays a pivotal role in acute myeloid leukemia (AML), depicting three different putative points of attack: differentiation arrest, bone marrow niche dependency of leukemia initiating cells (LIC), and acquired therapy resistance.

HA as well as CD44 antibodies can induce reverse of the leukemic differentiation blockage in a dose and time dependent manner, showing the strongest effect in monoblastic AML FAB M5 cells. The CD44 antibodies H90 and A3D8 as well as HA-12, an oligosaccharidic

fragment, and HMW-HA enable AML blasts to produce oxidative burst, increase expression of the differentiation specific markers CD11b, CD14, and CD15, and induce cytological changes confirming proceeding differentiation. This *in vitro* setting indicates that abrogation of the leukemic block might be a therapeutic strategy not only in AML FAB M3 and identified H90 and A3D8 as potential agents [95]. In combination with retinoic acid (RA) both antibodies overcome the maturation deficiency in the KG1a (FAB M0) cell line that is characterized by resistance to most differentiation-inducing agents. This depicts the possibility of synergistic effects of several agents to overcome the leukemic block in AML [96]. Molecular mechanisms behind the differentiation process have been identified in parts as e.g. in primary AML FAB M2-5 cells. Here anti-CD44 mAb treatment with H144a resulted in reduced *c-myc* transcript expression. Additionally seen increased differentiation and apoptosis was assumed to be responsible for this effect. [97]. Further THP-1 cell differentiation proceeds via autocrine cytokine secretion upon CD44 ligation. Hereby anti-CD44 mAb H3 treatment induced ERK1/2-mediated TNF-alpha and IL-6 secretion. Subsequent cell differentiation was shown to be dependent on both, TNF-alpha as well as IL-6. [67]. In primary AML FAB M5 cells, CD44-mediated GM-CSF and IL-8 secretion was identified to promote differentiation upon anti-CD44 mAb P245 treatment [68]. These findings indicate CD44-mediated stimulation of autocrine cytokine secretion to be a general mechanism for induction of differentiation in AML cells.

IL-8 and GM-CSF also mobilize hematopoietic stem cells (HSC) from BM into peripheral blood [98]. Differentiation and proliferation of HSC is closely connected to the microenvironment. Therefore they are dependent on finding their specific niche in the BM [99, 100]. Together with observed eradication of LIC and reduced repopulation in immune deficient mice [101], this might provide a way of antibody-triggered, CD44- and IL-8/GM-CSF-mediated targeting of AML cells via combined effect of reversed differentiation block and dependency of LIC on their bone marrow niche. Co-culturing of M6-AML TF-1 cells with mouse bone marrow-derived MS-5 cells resulted in increased CD34+ cell number and upregulation of CD44s and CD44v10. Anti-CD44 mAb 5F12 treatment displayed reduced adherence of TF-1 cells to the stroma [102].

In acute promyelocytic leukemia (APL) one molecular mechanism behind all-trans RA (ATRA)-resistance is the absence of functional CD44 expression on the cell surface and consequent apoptosis resistance: In the APL cell line NB4 treatment with anti-CD44 antibodies inhibited cell growth and induced apoptosis. The ATRA-resistant subclone NB4-LR1 displayed no CD44 expression due to epigenetic silencing mechanisms, which can be reversed treating the respective line with the DNA methylating inhibitor 5-aza-CdR. A similar effect is seen upon treatment with cyclic AMP and subsequent CD44 ligation by the anti-CD44 specific antibody A3D8, which results in apoptotic cell death [103].

CD44v6 surface expression correlates with shorter overall survival [19]. The t(8;21) translocation in AML is associated with upregulated CD44 both on mRNA and protein level[104].

Aggressive non Hodgkin lymphoma

Aggressive non Hodgkin lymphoma (NHL) show a new role of CD44 in diagnostic use, providing a model for minimal residual disease (MRD) detection and for discrimination between *c-myc*-associated (Burkitt lymphoma) and non-*c-myc*-associated lymphoma (Diffuse large B cell lymphoma: DLBCL). Further CD44v6 displays strong prognostic potential.

Examining 39 cases of childhood Burkitt lymphoma for CD44 surface expression by flow cytometry, deficient expression compared to normal B cells of similar maturity is observed. This characteristic possibly allows detection of tumor cells at a sub-microscopical level [105].

So far two distinct antibody panels for distinguishing between *c-myc*-rearranged and non-rearranged tumors have been introduced: CD44, CD38, and T cell leukemia 1 (Tcl-1), showing superiority to conventional staining with CD10 and Bcl-2 [106], and combination of CD44 and CD54 [107].

Among others Stauder et al examined a pool of 138 patients, including 76 low and 62 high grade NHL, showing an association between the expression of v6-containing isoforms of CD44 and aggressive NHL. Additionally strong expression levels of CD44v6 were highly significant correlated to a decreased overall survival but not to other prognostic markers as age and Ann Arbor classification determined in both groups. Neither ECOG performance status, extranodal involvement and serum LDH levels in the group of high grade NHL showed any association to CD44v6, indicating CD44v6 to be an independent factor for risk stratification [108, 109].

In DLBCL prognostic value of CD44 is well-documented: In primary nodal DLBCL, CD44s and CD44v6 expression levels are associated to tumor dissemination and survival. In a cohort of 276 patients with DLBCL CD44s as well as CD44v6 expression was correlated to tumor spread. Further CD44s displayed a strong predictive value for tumor related death independent to other parameters of the International Prognostic Index (IPI) [110]. Contrarily in another study of 46 patients with primary nodal DLBCL only CD44v6 expression was significantly correlated to poorer overall survival. While CD44v6 expression was predominantly observed in lymphoma cells, CD44s expression was also seen in non-neoplastic small lymphocytes [111]. Tissue microarray analysis of 90 DLBCL patients revealed correlation of disease stage to CD44v6 expression, and inverse correlation to

CD44s expression. In CD44s negative cases CD44v6 expression was associated with poorer overall survival.[112].

Examining 114 cases of mature B cell NHL, including gastric, nongastric extranodal, nodal follicular lymphomas, and nodal DLBCL, a novel translocation t(11;14)(p13;q32) juxtapositioning the regulatory 5' region of CD44 to the IGHS μ enhancer was indentified. This translocation resulted in the overexpression of a CD44 variant lacking Exon 1 [113].

Chronic lymphocytic leukemia

CD44v surface levels in chronic lymphocytic leukemia (CLL) are associated with advanced disease, therapy requirement, and lower median survival [114]. Unlike other surface molecules associated with other adhesion molecules like CD11a, CD49d, beta 1-3 integrins, CD54, CD58, and L-selectin, only the expression of CD44 and CD11c is associated with splenic manifestation of the disease. [115]. Soluble CD44 is related to shorter time of progression free survival [116], and soluble CD44s as well as soluble CD44v6 correlate with extended lymph node involvement, advanced Binet and Rai stage, and chemotherapy requirement [117].

As observed in other entities, CD44 displays an anti-apoptotic effect in CLL cells: In vitro data showed a protective effect of co-culturing CLL- with HK cells, a follicular dendritic cell line, against spontaneous apoptosis by increased levels of the anti-apoptotic Bcl-2 family member Mcl-1. In this setting, blockage of CD44 by antibodies resulted in Mcl-1 down-regulation and inhibition of the protective effect through the HK cells, indicating the anti-apoptotic mechanism being CD44-dependent [118].

Complex formation of CD44v, VLA-4, and proMMP-9 in CLL but not in normal B cells as well as increased proMMP-9 secretion upon CD44 antibody treatment provide a putative molecular mechanism of regulation of invasiveness. The functional relevance has not been further elucidated [119].

Multiple myeloma

In human myeloma derived-cells CD44 is shown to be involved in adherence to BM stroma cells (BMSC) and subsequent IL-6 secretion of latter [120]. Plasma cell lines reveal CD44v3, v6, and v9 expression regulating binding capacity of the plasma cells to stroma cells. CD44v9-mediated binding triggers IL-6 secretion of BMSC together with subsequent cell growth. Additionally loss of IL-6-dependent proliferation in XG-1 cells coincidences with loss of CD44v9 expression [121]. In multiple myeloma (MM) cell lines adhesion is dependent on CD44v6 expression and is upregulated by contact of the MM cells to BM endothelial cells

[122]. Further OPN induced proliferation as well as migratory capacity of MM cells is inhibited by anti-CD44v6 antibody [123].

High CD44v9 expression is associated with advanced disease stage, progressive disease, and shorter overall survival but with no other prognostic markers as serum lactic acid dehydrogenase (LDH) or beta2-microglobulin. This indicates CD44v9 to be an independent prognostic marker [124]. Further acquired CD44v9 expression occurs during disease progression [125]. CD44v6 expression is associated with chromosome 13q14 deletion and advanced disease stages in MM [126].

Hodgkin lymphoma

Resistance to apoptosis in Hodgkin lymphoma (HL) might be conferred by CD44/MIF interaction. CD44 and MIF are secreted in HL cell lines, expressed in primary HL cells, and increased in plasma of HL patient. According to the previous findings of MIF blocking the cytotoxic T lymphocyte (CTL) response and CD44 being essential for MIF/CD74 complex signalling, this interaction has been proposed as a potential strategy of the malignant cells to evade cytotoxic killing [127]. Another hypothesis provided suggests tissue inhibitory of metalloproteinase 1 (TIMP1) in cooperation with CD44 to rescue pre-apoptotic defect B cells in B cell malignancies as HL [128].

The CD44v10 isoform displays prognostic relevance by being correlated to initial BM involvement and risk of relapse in nodular sclerosing HL [129].

Experimental antagonization and drug resistance

For targeting the CD44 function in hematological malignancies, two mechanisms are worth considering: (i) direct targeting of CD44 and its variant isoforms expressed on the malignant cells as a primary therapeutical approach, and (ii) reversing an acquired resistance to primary treatment mediated through altered CD44 expression patterns by antagonizing of CD44 function.

Direct targeting of tumor cells by CD44 can be achieved by antibody based immunotherapy, eventually in context with labeling with radioactive substances or cytotoxic agents.

CD44v6 antibodies have been investigated extensively in squamous cell carcinoma (SCC). In radioimmunotherapy (RIT) of patients with metastatic and refractory disease, an anti-tumor effect was observed [130-133]. In the same setting, the CD44v6 antibody bivatuzumab has been used as vehicle for mertansine, a very strong inhibitor of microtubule assembly. Treatment related death of one patient resulted in no further clinical testing of the agent [134-

136]. Bivatuzumab mertansine failure in the clinical setting might be due to the high toxicity of mertansine itself. Being of 100- to 1,000-fold higher cytotoxic potency than other clinically used anticancer drugs such as taxanes or anthracyclines it might have been too potent for this kind of use.

Approaches of using ^{186}Re -labeled bivatuzumab in early stage breast cancer patients display limited efficiency because of its higher uptake levels in blood and bone marrow cells than in tumor cells. [137]. This in turn might indicate a promising role in treatment of hematological malignancies. Besides of the therapeutic effect also diagnostic use of CD44v6 antibodies is shown by successful detection of HNSCC lymph node metastases via immuno-PET by ^{89}Zr -cmAb U36 [139, 138].

Using HA, the natural binding partner of CD44, several drugs have been coupled to HA to be transported selectively to transformed cells: HA-But, a hyaluronic acid esterified with butyric acid, showing promising effects on malignant lesions of the liver [140], HA-cross-linked cisplatin displaying superior pharmacokinetics and pharmacodynamics to free cisplatin [141], HA-containing liposomes carrying mitomycin C [142], and paclitaxel targeting the malignant cells via CD44/HA interactions in cell lines [143] and in a human ovarian carcinoma nude mouse xenograft model. [144]. Retroviral gene delivery resulting in enforced CD44s expression and CD44v7-10 knock down experiments suggest CD44 alterations to be a possible target for gene therapy [145].

Multi drug resistance is a major problem in hematological malignancies. One mechanism behind this process is efflux pumps as for example the phosphoglycoprotein (Pgp) MDR1. In murine lymphoma and human leukemic cell lines decreased drug resistance achieved by treatment with HA oligosaccharides (oHA) is shown to be dependent on CD44 as the effect could be blocked by an anti-CD44 antibody. These findings indicate an important role of CD44 in mediating the efflux blocking effect of oHA on the Pgp transporter protein [146].

In DLBCL co-expression of CD44v and HA-mediated motility receptor (RHAMM) leads to unfavorable outcome when treated with cyclophosphamide, doxorubicin, vincristine, and prednisone [147]. Similarly in MM, treatment success of Dexamethasone was dependent on CD44 with decreased apoptosis rates in cells with high CD44 [148]. Here neutralizing CD44 might reveal new therapeutic strategies in treatment of multidrug resistant haematological malignancies.

At least two potential mechanisms have been provided in solid tumors as well as in inflammatory disease and might be useful models for treatment of haematological neoplasias: (i) Decrease of treatment resistance and increase of apoptosis in mesothelioma cell lines was achieved by siRNA silencing of CD44 or using CD44-neutralizing antibodies [149]. (ii) Developed for treatment of inflammatory disease PF-03475952, a fully human IgG2

anti-CD44 monoclonal antibody, might demonstrate a novel agent also useful in cancer therapy. By binding to CD44 it leads to inhibition of HA binding and thereby causes a loss of CD44 on the cell surface. The putative effect on drug resistance would have to be elucidated further in tumor settings [150].

Furthermore, new Imatinib-resistant CML cell lines display independency to common reported modes of resistance such as mutations of the bcr-abl domain, Lyn, Hck or MDR-1 overexpression. Instead CD44 and Fyn are upregulated in these cells and knock down or inhibition of Fyn results in re-sensitization to Imatinib [151]. Because of known CD44/Fyn association [53], the role of CD44 in this process has to be elucidated further.

Conclusions and perspective

By interaction with various different, and in some extent functional antagonistic proteins, CD44 seems to take part in a wide range of distinct cell functions. Because of its regulatory activity concerning e.g. cell migration, invasion, survival, and growth, altered CD44 expression might be an important step towards malignant transformation. This underlines the importance of understanding the molecular mechanisms behind the obvious effect of the different CD44 expression pattern in distinct tumor entities.

There have been a lot of previous research approaches concerning CD44 in solid tumors. Here we focused on hematological neoplasias where CD44 has three main functions: First its role as prognostic marker, second its potential role for diagnosis, and third its role as a promising therapeutic target.

Distinguishing between different prognostic subtypes of one neoplastic disease entity is of great advantage. E.g. this aspect is of eminent importance in ALL because of the very young median age of the patients on the one hand and the very bad prognosis of a disease relapse on the other hand. For preventing secondary malignancies induced by too aggressive treatment regimes a deliberate risk stratification based on the expected tumor prognosis is imperative. Another example gives the observed treatment resistance to several standard therapeutics associated with CD44 expression in a limited group of patients with DLBCL. This provides the possibility of improved individual treatment decisions, which have been a big aim in cancer therapy in the last years.

More and more, CD44 is considered being of diagnostic use. First approaches were made in Burkitt lymphoma, where it was suggested to detect malignant cells on a sub-microscopical level by flow cytometry. In addition CD44 was claimed to be able to distinguish, together with other antibodies, between *c-myc*-associated lymphoma and DLBCL, which is of great importance concerning treatment decision.

The last function might be the most promising. Several approaches have been made investigating the possible role of CD44 in new therapy strategies of solid tumors. CD44 antibodies as well as HA achieved promising results in therapy and diagnostic. Also first research achievements were made concerning CD44 and gene therapy, opening a wide interesting field for future investigation. The development of CD44 targeted therapeutic concepts in hematopoietic malignancies is far behind compared to treatment of solid tumor entities but latter can be used as model for future approaches in hematological neoplasias. Furthermore overcoming acquired treatment resistance in hematological malignancies certainly needs to be investigated in the near future.

Table 1. Extracellular CD44 binding partners

Ligand	Interaction	Reference
HA	CD44/HA interactions enhance cell motility, proliferation, and survival	Among others Torre et al. [39]
	HA ligation of CD44 is able to increase cell adhesion and differentiation	Among others Bourguignon et al. [58]
sOPN	Binding to CD44 stimulates cell survival	Lin and Yang-Yen [64]
RHAMM	Cooperates with CD44 and enhances cell motility	Hamilton et al. [152]
VLA-4	Interacts with CD44 and mediates adhesion of hematological progenitor cells	Verfaillie et al. 1994 [35]
	Cooperates with the cytoplasmic tail of CD44 and assures firm cell adhesion and cell extravasation	Nandi et al. [36]
	CD44 increases VLA-4 expression and enhances transendothelial migration of tumor cells	Wang et al. [38]
LFA-1	CD44 increases LFA-1 expression and enhances transendothelial migration of tumor cells	Wang et al. [38]
Galectin-8	Binding to CD44 induces apoptosis	Eshkar-Sebban et al. [153]
Galectin-9	Binding to CD44 stimulates CD44/BMP receptor II complex formation and induces osteoblast differentiation	Tanikawa et al. [49]

Table 2. Extracellular CD44/Growth factor interactions

Ligand	Interaction	Reference
IGF-1	Induces CD44v6 surface expression in human neuroblastoma cell lines and increases HA-binding activity	Fichter et al. 1997 [6]
TGF-beta	Is activated by CD44/MMP-9 complex and leads to enhanced tumor cell survival	Yu et al. 2004 [54]
	Stimulates CD44/ErbB1 co-localization and fibroblast differentiation	Simpson et al. [69]
FGF-4/FGF-8	Bind to heparan sulphate modified CD44v3 and stimulates limb mesenchymal cell proliferation	Sherman et al. [154]
PDGF	Enhances CD44v transcription and increases HA-binding affinity	Fichter et al. [6]
HGF	Activates c-Met via the extracellular part of CD44v6 and is required for cell motility in human melanocytes	Orian-Rousseau et al. [155], Recio et al. [156], Damm et al. [157]
HB-EGF	Binds to heparan sulphate modified CD44v3 and leads to enhanced cell survival.	Bennett et al. [158], Yu et al. [159]
EGF	Up-regulates CD44s expression and thereby increases invasiveness of astrocytoma cells	Monaghan et al. [160]
	Promotes CD44 cleavage and cell migration	Murai et al. [17]
VEGF-A	Leads to CD44v6-dependent angiogenesis	Tremmel et al. [161]

Table 3. CD44/receptor tyrosine kinase interactions

Protein	Interaction	Reference
ErbB1	Upon TGF-beta stimulation co-localizes with CD44 and results in ERK1/2 phosphorylation and activation	Simpson et al. [69]
ErbB2	Forms complex with CD44, resulting in HA-induced kinase activity	Bourguignon et al. [162]
	Activated by CD44/HA, ErbB2 phosphorylates beta-catenin which thereupon translocates into the nucleus, stimulating LEF-1/TCF-4 transcriptional activity	Bourguignon et al. [163]
ErbB4	Phosphorylated by CD44v3/HB-EGF complex	Yu et al. [159]
VEGFR-2	Activated by CD44v6 acting as co-receptor	Tremmel et al. [161]
TGF-beta RI	Co-localized with CD44 in HK-2 cells	Ito et al. [60]
BMP-RII	Forms a complex with CD44 and leads to downstream smad phosphorylation	Tanikawa et al. [49]
c-Met	Is phosphorylated by CD44v6/HGF and triggers intracellular signaling via NF-kappaB and the transcription factors Egr-1 and C/EBP-beta. This leads in turn to enhanced CD44v6 expression.	Recio et al. [156], Damm et al. [157]

Table 4. Impact of CD44 signalling on MMPs

Protein	Interaction	Reference
MMP-1	Expression influenced by CD44/chondroitin sulfate proteoglycan (CSPG) ligation	Baronas-Lowell et al. [40]
MMP-2	Upregulated by CD44 antibody resulting in enhanced invasiveness of human melanoma cells	Takahashi et al. [164]
MMP-7	Is recruited to the membrane by CD44v3 and activates pro-HB-EGF.	Yu et al. [159]
MMP-8	Expression promoted by CD44/CSPG ligation	Baronas-Lowell et al. [40]
MMP-9	Forms complex with CD44, leading to retained MMP-9 proteolytic activity, associated with collagen IV degradation and enhanced invasiveness	Yu et al. [41]
	Activates TGF-beta in cooperation with CD44, leading to increased survival	Yu et al. [54]
	Co-expressed with CD44v4 in advanced breast cancer stages	Thanakit et al. [165]
MMP-13	Expression promoted by CD44/CSPG ligation	Baronas-Lowell et al. [40]
	Secretion inhibited by CD44/HA interference	Julovi et al. 2010 [166]
MMP-14	Expression modulated by CD44/CSPG ligation	Baronas-Lowell et al. [40]
MT1-MMP	Processes CD44, resulting in sCD44 release and tumor cell migration	Kajita et al. [15]

Table 5. CD44-mediated cytokine expression

Cytokine		Cell type	Reference
IL-1	Release upon CD44 ligation	Monocytes	Denning et al. [167]
IL-1 alpha	Increased gene expression after CD44 ligation	AML FAB M5 blasts	Delaunay et al. [167]
IL-1 beta	CD44 Ligation induces enhanced gene transcription and secretion	AML FAB M5 blasts	Delaunay et al. [68]
IL-6	Secretion regulated by CD44v	Macrophages	Khaldoyanidi et al. [72]
	Secretion induced by CD44v9 antibody	Plasma cell lines	Van Driel et al. [121]
	Increased synthesis after CD44 ligation in THP-1 monoblastic leukemia cells	AML cell line AML FAB M5 blasts	Bourcier et al. [67]
	CD44 Ligation induces enhanced gene transcription and secretion		Delaunay et al. [68]
IL-8	IL-8 secretion mediated by CD44	Macrophages	Mc Kee et al. [86]
	CD44 Ligation induces enhanced gene transcription and secretion	AML FAB M5 blasts	Delaunay et al. [68]
IL-10	Expression inhibited by CD44/OPN interactions	Macrophages	Ashkar et al. [83]
IL-12	Increased gene expression after CD44 ligation	AML FAB M5 blasts	Delaunay et al. [68]
IL-13	Increased gene expression after CD44 ligation	AML FAB M5 blasts	Delaunay et al. [68]
TNF alpha	Synthesis triggered by CD44 ligation in THP-1 monoblastic leukemia cells	AML cell line	Bourcier et al. [67]
GM-CSF	CD44 Ligation induces gene transcription and secretion	AML FAB M5 blasts	Delaunay et al. [68]

Figure 1. Exon organization

Dark green circles represent the constant exons, light green the variable ones (v1-10). The extracellular part (EC) is encoded by exon 1 to 17 (E1-17), spanning at least 248 amino acids: E1-5 form the amino terminal globular region, E16 and 17 encode the stem (46 amino acids) with possible insertion of v1-v10, whereas v1 is not existent in the human genome. E18 represents the transmembrane region (TM) that consists of 23 hydrophobic amino acids and a cysteine residue. E19 or E 20 encode the intracellular (IC) cytoplasmic tail, creating either a short tailed (3 amino acids) or a more abundant long tailed (72 amino acids) CD44.

Figure 2. Protein structure

CD44v (left) compared to CD44s (right). Globular extracellular region includes HA binding motifs (yellow), one located inside the so called 'link domain' spanning amino acids 32 to 123, and the other at amino acid positions 150 to 158, and a cleavage site (red). Exon v3 can be modified by heparan sulphate (purple). The cytoplasmic tail embodies a basic amino acid binding site for ERM and Merlin at amino acid 292 to 300 (dark blue), an ankyrin binding site at amino acid 304 to 318 (light blue), and a Lck binding site located at the membrane proximal region.

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