Abstract. CD147 is involved in many physiological functions, such as lymphocyte responsiveness, spermatogenesis, implantation, fertilization and neurological functions at early stages of development. Here we specifically review the role of CD147 in cancer. We focus on the following aspects: expression of CD147 in malignant versus normal tissues and its possible impact on prognosis, interaction of tumor cell-expressed CD147 with stroma cells and induction of matrix metalloproteinases, as well as the role of CD147 in tumor angiogenesis. The function of CD147 in supercomplexes with monocarboxylate transporters (MCT) and amino acid transporters such as CD98hc and large neutral amino acid transporter 1 (LAT1), as well as the functional contribution of CD147 in complexes with caveolin-1 and integrins, is discussed. Finally, the relevance of CD147 as a target for therapeutic intervention in cancer patients is discussed.

CD147 is a member of the immunoglobulin family of receptors. Members of this family play a role in intercellular communication involved in many immune-related functions, differentiation and development. CD147 plays a role in spermatogenesis, lymphocyte activation, expression of monocarboxylate transporters (MCT) and has been identified as a regulatory subunit of the γ-secretase complex in Alzheimer’s disease amyloid β-peptide production (1-5). Some of these insights were obtained from the study of cd147−/− mice. These animals are defective in matrix metalloproteinase (MMP) regulation, spermatogenesis, lymphocyte responsiveness and neurological functions at the early stages of development. Such female mice are infertile due to failure of implantation and fertilization (5). CD147 is involved in the transport of the MCT-1 and MCT-3 to the plasma membrane since reduced accumulation of these transporters has been observed in the retina of cd147 knockout mice. A functional role of CD147 in cell adhesion is supported by its involvement in the blood-brain barrier and its interactions with integrins. CD147 has been implicated in many pathological processes, such as rheumatoid arthritis, experimental lung injury, atherosclerosis, chronic liver disease induced by hepatitis C virus, ischemic myocardial injury and heart failure (4). Treatment of transplant patients with a CD147 antibody was effective due to inhibition of T-cell activation (6). In the following pages, we review the expression and the functional role of CD147 in human cancer and discuss its possible role as a target for therapeutic intervention.

General Features of CD147

CD147, a transmembrane protein of the immunoglobulin (Ig) superfamily was identified independently in different species and has many designations across different species such as M6, Neurothelin, SA11, HT7, OX-47, CE9, EMMPRIN, Basigin, and gp42 (7-11). The most prevalent standard isoform is a single-chain type I transmembrane molecule composed of a 21 amino acid signal sequence, a 186 residues-long extracellular domain consisting of two Ig-like domains, a transmembrane domain of 21 amino acids and a cytoplasmic domain of 41 residues. The topology of CD147 and a rarely occurring splice variant as well as the corresponding amino acid sequences are shown in Figures 1 and 2. The transmembrane region harbors a leucine zipper and a charged residue (glutamic acid). The corresponding
gene is located on chromosome 19p13.3 and encodes a 29 kDa backbone protein. Three N glycosylation sites have been identified and migration on sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) occurs between 39 and 65 kDa depending on the degree of glycosylation. A CD147 transcript as a new alternative splice variant composed of three Ig-like domains has been identified in the retina of the mouse (12). This isoform, Figure 1 right, results from the translation of a region within the first intron of CD147 as indicated in Figure 2 for human CD147. This splice variant was shown to interact homophilically and might function in alignment of lactate transporters in the retina where lactate is an important metabolite.

CD147 has a broad expression pattern on hematopoietic and non-hematopoietic cells such as monocytes, granulocytes, epithelial and endothelial cells. Weak expression has been noted on resting T lymphocytes, whereas expression is increased on activated T lymphocytes and monocytes (7-11, 13). Amino acid-based sequence comparisons of the CD147 orthologs of chicken, mouse, rat, rhesus monkey, chimpanzee and human are shown in Figure 3. The amino acid sequence homology of the human protein versus that of chimpanzee, rhesus monkey, rat, mouse and chicken is 96%, 88%, 65%, 65% and 53%, respectively. The perfect conservation of the amino acid sequences of the transmembrane sequences across all listed species (2) as described above is a remarkable feature, which includes the presence of a conserved glutamic acid residue. This finding indicates the involvement of transmembrane amino acids in protein-protein interactions within the plasma membrane. The cytoplasmic domains are more conserved than the extracellular domains pointing to similar considerations with respect to conserved protein-protein interactions with proteins located in the cytoplasm.

CD147 paralogs are embigin, a developmentally expressed protein (14), and neuroplastin, which acts as a synaptic glycoprotein (15). The amino acid sequence alignment of CD147 with its paralogs is shown in Figure 4, indicating amino acid sequence homology between human CD147, embigin and neuroplastin as 22% and 36% respectively. It is again noteworthy that the strongest amino acid conservation among the paralogs is observed in the transmembrane domains, including the charged glutamic acid.

**Induction of MMP Is a Cancer-related Feature of CD147**

It has been shown that CD147-positive tumor cells and their supernatants induce expression of MMPs such as MMP-1, MMP-2, MMP-3, MMP-9 and MMP-11, in cultured fibroblasts (16-18). Therefore, CD147 is also designated as extracellular MMP inducer (EMMPRIN). It was shown that MMP induction can also be mediated by soluble CD147, which was found in tissue culture supernatants as full-length protein or as a protein containing the extracellular domain only generated by MMP-mediated shedding (19-21). Soluble CD147 also has been detected in microvesicles (exosomes) (22, 23). Homotypic interactions may play an important role regarding the mode of action of MMP induction since it has been shown that recombinant CD147 fusion proteins can interact homotypically (24). However, interaction of CD147 with an as yet unidentified receptor might also be responsible for the phenomena as described above. CD147 can also be induced in tumor cells by epidermal growth factor receptor (EGFR)-mediated signaling (autocrine mechanism) (25). Controlled degradation of the extracellular matrix is a prerequisite for tumor invasion and metastasis. Transfection of breast cancer cells with CD147 resulted in increased tumor growth and metastasis after implantation into the mammary gland and correlated with high levels of MMP-2 and MMP-9 (26). The molecular mechanisms of these phenomena have been poorly resolved. Glycosylated CD147 was shown to be a prerequisite for MMP induction at the transcriptional level (25). Induction of MMPs by CD147 was prevented with a monoclonal antibody directed against CD147 (25).

**CD147 Mediates MMP-dependent and -independent Angiogenesis**

It was found that CD147 expression was significantly up-regulated in activated human umbilical venule endothelial cells (HUVEC’s) (27). Inhibition of CD147 expression by RNAi led to significantly decreased angiogenesis in vitro. CD147 may regulate angiogenesis by several mechanisms including proliferation, survival, MMP secretion and phosphoinositide 3 kinase/protein kinase B (PI3K/Akt) activation. Modulation of remodeling of the extracellular matrix by MMPs and its impact on angiogenesis is a well-known phenomenon. In addition, it was shown that CD147 is involved in the induction of vascular endothelial growth factor (VEGF) (28). In the B16 melanoma model, MMP-2/MMP-9 expression occurs independently of CD147 (28). Knock-down of CD147 caused reduced VEGF production in vivo accompanied by reduced blood vessel formation, supporting the notion that CD147 promotes MMP-independent angiogenesis in this model. CD147 stimulates VEGF production in tumor and stromal compartments and VEGF induction involves the PI3K/Akt pathway. CD147 was shown to be required for responses to bevacizumab therapy in head-and-neck squamous cell carcinoma models (29). Bevacizumab therapy was effective in FaDu xenografts expressing CD147, but not in tumors with silenced CD147 expression. Tumor vesicle-associated CD147 was shown to modulate the angiogenic activity of HUVECs. This was
Figure 1. Topology of human CD147 isoforms. The most frequently expressed variant of CD147 (2 Ig-like domain form) is shown on the left, the rarely expressed CD147 variant (3 Ig-like domain form) is shown on the right. Modules, N-glycosylation sites, phosphoserine residues and isoform-specific amino acids are highlighted according to the Swissprot entry BASI_HUMAN.

Figure 2. Amino acid sequence and functional motifs of human CD147. Domains and motifs are highlighted by a color code as outlined. The positions of the Ig-like domains are labeled according to Interpro Prosite PROFILE PS50835, that of the first Ig-like domain and the disulfide bridges according to Interpro SMART SM00408. All other annotations are based on the Swissprot entry BASI_HUMAN.
Figure 3. Amino acid sequence alignment of CD147 from different species (orthologs). Identical amino acids are indicated by a light blue background. Ig-like C2-type domains, Ig-like V-type domains and the transmembrane domains are boxed by dark blue, green and black colors, respectively. Chimp and rhesus sequences are predicted based on their genome sequences, the other sequences were derived from the Swissprot database (BASI_X).
shown for vesicles derived from ovarian carcinoma cell lines OVCAR3, SKOV3 and A2780 (22). Treatment of OVCAR3 cells with RNAi directed against CD147 suppressed the angiogenic potential of OVCAR-3 derived microvesicles.

**CD147 Association with Cyclophilins (Cyps)**

Cyps are a family of proteins that share peptidyl-prolyl cis-trans isomerase activity which is involved in their chaperone function. However, there is also evidence that Cyps are involved in intracellular communication (30). Cyps are located intracellularly as well as extracellularly. It was shown that extracellular CypB might be involved in recruitment of CD4+ T-cells into infected tissue (31). Competition experiments have shown that CypA and CypB share a common receptor at the cell surface (32) which was identified as CD147 (33). CypA and CypB are able to transduce signals in several types of cells (34). Few reports covering cancer-related aspects of CD147/Cyp interaction are available. Expression of CD147 and CypA in pancreatic adenocarcinoma tissue are substantially higher than in corresponding normal tissues. Exogenous CypA significantly stimulated cancer cell proliferation in a dose-dependent manner and this effect was blocked by pretreatment with anti-CD147 antibody. In addition, CypA stimulated extracellular signal-related kinase 1 and 2 (ERK1/2) and p38 mitogen-activated protein kinase (MAPK) signaling pathways and increased the secretion of cytokines (IL5 and IL17) in Panc-1 cells. Several studies have shown the involvement of inflammatory or proinflammatory mediators in oncogenesis. IL5 is involved in the pathogenesis of atopic diseases and regulates production, activation and localization of eosinophils causing tissue damage in atopic diseases. IL17 stimulates secretion of chemokines containing nuclear factor κB (NFκB) binding sites in their promoter regions, including IL8 and monocyte chemoattractant protein-1 (MCP-1). A role of CypA in cancer is supported by the finding that CypA and macrophage inhibitory factor (MIF) are the most dominantly expressed proteins in non-small cell lung carcinoma (35) and a novel cyclophilin similar to CypA has been associated with metastasis and shown to be overexpressed in bladder cancer, hepatocellular carcinoma, sarcoma and breast carcinoma (36).

**Target Validation Experiments with RNAi Reveal Functional CD147/MCT Interactions**

CD147 has been described as an ancillary protein for function and expression of monocarboxylate transporters MCT1 (SLCT16A1), MCT3 (SLC16A3) and MCT4 (SLC16A4) (37-
Figure 5. Schematic overview of CD147-associated proteins, mediators of signaling and transcriptionally induced genes. Details are given in the text. HSPG, Heparan-sulfate proteoglycan; FAK, focal adhesion kinase; MEK, Map-Erk kinase; ERK, extracellular signal-related kinases; Akt, serine-threonine kinase; MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor; α3β1 and α6β1, integrins; MCT, monocarboxylate transporter; LAT1, large neutral amino acid transporter; CD98, subunit of LAT1; ASC2, Asc-type amino acid transporter-2.

Figure 6. CD147 neighbourhood analysis. Ingenuity pathway analysis (IPA) was used to create the neighborhood of genes for CD147. Shown are all molecules with a direct relationship to CD147. Edges are annotated by the relationship type of two molecules: PP, protein-protein interaction; E expression; P, phosphorylation; LO, location; PD, protein DNA interaction; TR, translocation; A, activation. All neighboring molecules of CD147 were manually grouped and annotated according to their molecular type. MMP1/2/3, matrix metalloproteinases 1/2/3; NCSTN, nicastrin; Mmp, group of matrix metalloproteinases; RFXANK, regulatory factor X-associated ankyrin-containing protein; STAT4, signal transducer and activator of transcription; RYR1, ryanodine receptor 1; PTPN11, protein tyrosine phosphatase, non-receptor type 11; NT5C3, 5'-nucleotidase, cytosolic III; SLC2A4, solute carrier family 2, member 4; SLC16A4, solute carrier family 16, member 4; SLC16A1, solute carrier family 16, member 1; ATP1B2, ATPase, Na⁺K⁺ transporting, beta 2 polypeptide; ATP1A2, ATPase, Na⁺K⁺ transporting, alpha 2 (+) polypeptide; SCP2, sterol carrier protein 2; AJAP1, adherens junctions-associated protein 1; MCT5, malignant T-cell amplified sequence 1; APP, amyloid beta (A4) precursor protein; RANBP3, RAN binding protein 3; ATXN10, ataxin 10; PDLIM7, PDZ and LIM domain 7; PRNP, prion protein; BAD, BCL2-associated agonist of cell death; PNN, pinin, desmosome-associated protein; BCL2L11, BCL2-like 11; PPIA/PPIC, peptidylprolyl isomerase A/C (cyclophilin A/C); LEP, leptin; CSF2, colony-stimulating factor 2; SPP1, secreted phosphoprotein 1; Pk(s), group of protein kinases c; ERK1/2, group of ERKs; ERK, group of ERKs; Akt, group of Akts; MAP2K1/2, mitogen-activated protein kinase kinase group of kinases.
Expression of MCT3 seems to be restricted to the retina, whereas MCT1 and MCT4 are broadly expressed in many tissues. Functional transporters are heteromers composed of CD147 and MCT1 or MCT4 and are involved in transport of lactate from the cytoplasm to the outside of the cell (41). Tumor cells are characterized by excessive anaerobic glycolysis resulting in deregulated lactate production, a phenomenon referred to as the Warburg effect (42).

Several groups have shown the impact of CD147 and its linkage to MCT with respect to cell viability, migration and proliferation by neutralization of CD147 function via knock down of its RNA or by inhibition of the protein with monoclonal antibodies. Blocking of CD147 with monoclonal antibody MEM-M6/1 (43) induces cell death in LoVo, HT-29, WiDr and SW620 colon cancer cells, and A2058 melanoma cells, but not in WI-38 and TIG-113 normal fibroblasts. MEM-M6/1 inhibits lactate release and lowered the intracellular pH. Induction of acidification correlated with inhibition of glycolytic flux and a decrease in intracellular ATP levels. In A375 human melanoma
cells, a CD147-targeting siRNA was shown to inhibit proliferation, invasiveness, VEGF production and down-regulation of glycolysis (27). CD147 RNAi knockdown in a human Jurkat T-lymphoma cell line (44) resulted in reduced proliferation, migration and reduced adhesion to the extracellular matrix protein fibronectin in vitro. Silencing of CD147 by RNAi reduced the proliferation rate of MiaPaCa2 and Panc1 pancreatic carcinoma cell lines (45). CD147 silencing reduced invasiveness through the chorioallantoic membrane of chick embryos (CAM assay) and inhibited tumorigenicity in a xenograft model in nude mice (45). These observations can be explained by an increase in intracellular lactate concentration resulting in cell death since lactate has been shown to lower pyruvate reduction to lactate by lactate dehydrogenase (LDH) and inhibition of LDH was shown to reduce the Warburg effect and to inhibit proliferation of tumor cells (46). The role of CD147 in stroma tumor interactions was studied with SW620 colon cancer cells (47). Experiments in vitro indicated that colon cancer stromal cell interactions mediated by CD147 led to increased MMP-2 expression in fibroblasts, but not in macrophages. CD147 RNAi targeting stroma cell CD147 was able to significantly down-regulate CD147 mRNA levels in mice bearing human colon cancer xenografts and suppress growth of the xenografts. These observations suggest that CD147 expressed on stroma cells plays an important role in growth of the SW620 colon cancer xenograft. Blockage of tumor expressed CD147 by RNAi did not result in inhibition of tumor growth. Proteomics-based analysis revealed further interaction partners of CD147 in addition to MCT: CD98 heavy chain (CD98hc), large neutral amino transporter 1 (LAT1) and Asc-type amino acid transporter 2 (ASCT2), all of which are L-type amino acid transporters (48). These interactions were revealed by covalent cross-linking and mass spectrometry. The data reveal the existence of a CD147-CD98 cell surface supercomplex that plays a role in energy metabolism, likely by coordinating transport of lactate and amino acids. CD98hc is a multifunctional glycoprotein with a single transmembrane domain, is highly expressed on proliferating cells and functions as a chaperone for amino acid transporters. It has been shown that CD98hc forms disulfide-bridged heterodimers with at least six types of L-type amino acid transporters (48). Co-regulation of cell surface CD98hc/LAT1 and CD147/MCT protein complexes support their physical interactions. In addition, interactions of CD98hc with integrin β1 and epithelial cell adhesion molecule (EpCAM) have been described (48, 49). Altogether, the identified interactions point to the existence of a CD147-based supercomplex. Interactions of CD147 with other proteins are summarized in Figures 5 and 6.

Association of CD147 with Caveolin and Integrins

CD147 associates with caveolin-1, thereby diminishing both CD147 clustering and CD147-dependent MMP-1-inducing activity (50). Highly specific association on the surface of several types of cells has been observed. Complex formation is temperature and cholesterol dependent, reminiscent of associations seen with caveolea/lipid rafts. Overexpression of CD147 caused a specific decrease in clustering of cell surface CD147. It was shown that caveolin interacts with low glycoforms of CD147 and inhibits their conversion to high glycoforms which are the isoforms which self-aggregate and stimulate MMP production (51). Interaction of CD147 with integrins α6β1 and α3β1 is responsible for the invasion potential of human hepatoma cells (52). α6β1 and CD147 co-localize on human hepatoma cells. The enhancing effect of CD147 on invasion capacity and secretion of MMPs was partially blocked by α6β1 antibodies. Wortmannin, a PI3K inhibitor that reverses the effect of CD147 on the regulation of intracellular Ca2+ mobilization, significantly reduced cell invasion potential and secretion of MMPs in human hepatoma cells. Based on these data, α6β1 interacts with CD147 to mediate tumor invasion and metastasis through the PI3K pathway. In addition, it was shown that in human hepatoma cell overexpression of CD147 promotes invasion and metastasis via α3β1 integrin-focal adhesion kinase (FAK)-paxillin and FAK-PI3K- Ca2+ pathways. α3β1 and CD147 co-localize on human 7721 hepatoma cells. The enhancing effect of CD147 on adhesion, invasion and MMP secretion was reduced by integrin α3β1 antibodies. Expression of integrin downstream molecules including FAK, phospho-FAK, paxillin and phospho-paxillin were increased in human hepatoma cells overexpressing CD147. Wortmannin and LY294002, specific PI3K inhibitors, reversed the effect of CD147 on the invasion of a human hepatoma cell line. CD147 promotes invasion and metastasis via α3β1 integrin. CD147 associates with caveolin-1, thereby diminishing both CD147 clustering and CD147-dependent MMP-1-inducing activity (50). Highly specific association on the surface of several types of cells has been observed. Complex formation is temperature and cholesterol dependent, reminiscent of associations seen with caveolea/lipid rafts. Overexpression of CD147 caused a specific decrease in clustering of cell surface CD147. It was shown that caveolin interacts with low glycoforms of CD147 and inhibits their conversion to high glycoforms which are the isoforms which self-aggregate and stimulate MMP production (51). Interaction of CD147 with integrins α6β1 and α3β1 is responsible for the invasion potential of human hepatoma cells (52). α6β1 and CD147 co-localize on human hepatoma cells. The enhancing effect of CD147 on invasion capacity and secretion of MMPs was partially blocked by α6β1 antibodies. Wortmannin, a PI3K inhibitor that reverses the effect of CD147 on the regulation of intracellular Ca2+ mobilization, significantly reduced cell invasion potential and secretion of MMPs in human hepatoma cells. Based on these data, α6β1 interacts with CD147 to mediate tumor invasion and metastasis through the PI3K pathway. In addition, it was shown that in human hepatoma cell overexpression of CD147 promotes invasion and metastasis via α3β1 integrin-focal adhesion kinase (FAK)-paxillin and FAK-PI3K- Ca2+ pathways. α3β1 and CD147 co-localize on human 7721 hepatoma cells. The enhancing effect of CD147 on adhesion, invasion and MMP secretion was reduced by integrin α3β1 antibodies. Expression of integrin downstream molecules including FAK, phospho-FAK, paxillin and phospho-paxillin were increased in human hepatoma cells overexpressing CD147. Wortmannin and LY294002, specific PI3K inhibitors, reversed the effect of CD147 on the regulation of intracellular Ca2+ mobilization and reduced cell adhesion, invasion and MMP secretion. In Drosophila melanogaster, CD147 promotes cytoskeletal rearrangements and the formation of lamellipodia. CD147 and integrin co-localize in cultured insect cells and in the visual system. The effect of CD147 is integrin-dependent as shown with argin-glycine-aspartate (RGD) peptides and mutation analysis (53, 54). In leukocytes, CD147 has also been shown to interact with the sialoglycoprotein CD43 and thereby regulates adhesiveness of leukocyte function antigen 1 (LFA-1), the broadly expressed β2 integrin of leukocytes (55).

Function of CD147 as an Anti-apoptotic Protein and Mediator of Chemoresistance

In several cancer cell lines, CD147 has been identified as a mediator of anti-apoptotic function and chemoresistance. In HO-8910 ovarian carcinoma cells, CD147 RNAi reduces
Expression of CD147 in Cancer

High incidence of expression of CD147 in different cancer entities making use of tissue microarrays and monoclonal antibodies (mAb) MEM-M6/1 and HIM6 (65) was noted in a systematic investigation (65). A lot of 2348 and 608 tissue samples covering 129 distinct tumor types and 76 different normal tissues, respectively, were investigated for their CD147 status with these antibodies. CD147 expression was found in 112 out of 129 tumor entities with the following incidences: squamous cell carcinomas (60-100%), pancreatic (87%), chromophobous kidney (83%), hepatocellular (83%), medullary breast (83%) and glioblastoma multiforme (79%). Homogeneous expression of CD147 was found in tumor types such as squamous cell carcinoma of different types of organs and mesotheliomas. The following normal tissues scored positively for CD147 expression: proliferatively active and differentiating epithelial cells, myocardial cells in the left heart ventricle and vascular endothelial cells of the brain. Interestingly, CD147 isoforms differing in presence or absence of Lewis X glycan structures were found on breast cancer cells.

Another investigation of expression and function of CD147 as a cancer-associated biomarker made use of mAb HIAb18 (IgG1) (66). A lot of 28 tissue microarrays and 1117 pathological sections of breast tissue samples were analyzed. The incidence of CD147 expression was: cancer of the liver 80% (n=20), lung 62% (n=90), stomach 66% (n=44), colon 58% (n=19), rectum 59% (n=17), breast 64% (n=1055), cancer 80% (n=10), brain 90% (n=52), oesophagus 87% (n=16), ovary 75% (n=40), urinary bladder 85% (n=41), skin (squamous cell carcinoma) 58% (n=41), larynx 85% (n=63) and kidney 73% (n=33), and 30% of sarcomas such as osteo, chondro- and fibrosarcoma (n=102). Staining was ranked as weak, moderate and strong. Strong staining was observed in 20% of breast, ovarian and brain tumors. In a retrospective study of 106 patients with infiltrating ductal carcinoma of the breast, the level of CD147 expression was correlated with survival of the patients (66).

Up-regulation of CD147 also has been noted in glioma, laryngeal squamous cell, ovarian, renal cell and skin carcinoma (67-71). CD147 was described as a marker of poor diagnosis in serous ovarian and bladder carcinomas (72, 73).

We have analyzed RNA-based CD147 expression in liver and bladder cancer in comparison to corresponding normal tissues based on GEO database expression data (74). As shown in Figure 7A, which includes normal liver, low- and high-grade dysplastic liver tissue, cirrhotic liver as well as early and advanced hepatocellular carcinoma, RNA levels of CD147 in the liver pathologies are significantly higher in comparison to normal liver tissue and correlate with pathogenicity. Analogous findings are presented for bladder carcinoma in Figure 7B. Steady-state RNA levels of CD147 are increased in transitional bladder carcinoma compared to normal bladder tissue and carcinoma in situ.

Antibodies Directed against CD147

Many mAbs directed against CD147 interacting with distinct epitopes have been established (75). Most of the antibodies only bind to phytohemagglutinin (PHA) stimulated T-cells, not to resting T-cells. This phenomenon was explained by bivalent binding of the low-affinity antibodies to clustered CD147 molecules on activated T-cells and not by neo-epitopes specifically displayed on activated T-cells. High affinity antibodies were able to bind in a monovalent fashion to resting T-cells, which are low expressors of CD147. Induction of dimerisation by the low-affinity antibodies resulted in inhibition of CD3-mediated T-cell activation. High-affinity mAb MEM-M6/6, recognizing a unique epitope, inhibits T cell activation by 80% and as outlined in...
a previous section also proliferation of colon cancer and melanoma cells and not non-transformed fibroblasts (43). Triggering of CD147 by mAbs was shown to cause displacement of glycosylphosphatidylinositol (GPI)-anchored co-receptors CD48 and CD59 from microdomains in human T-lymphocytes (76). Perturbation of microdomains is responsible for inhibition of T-cell proliferation. Making use of COS-7 transfectants and mAbs covering different CD147 epitopes, it was shown that CD147 contains different epitopes involved in regulation of cell adhesion (homotypic cell aggregation) and lymphocyte activation (77).

A different set of CD147-directed mAbs were evaluated with respect to treatment of hepatocellular carcinoma (HCC) (78). mAb Hb18G and Licartin, a 131I-labeled F(ab')2 fragment of mAb Hb18G mediate suppression of MMP secretion in cocultured fibroblasts and inhibit invasion, and Licartin significantly inhibited the growth of HCC cells. mAb Hb18G and Licartin effectively reduced growth and metastasis as well as the expression of stromal factors such as MMPs, VEGF and fibroblast surface protein (78). Clinical studies were reported for targeted radioimmunotherapy of HCC patients with Licartin (79). Of the 73 patients completing two cycles in a phase II trial, 6 (8%) were noted to have a partial response, 14 (19%) a minor response and 43 (59%) had stable disease. The survival of progression-free patients was significantly higher than that of patients with progressive disease. Chimeric CD147 antibody (IgG1) referred to as CNTO 3899 was evaluated as an agent for potential treatment of head-and-neck squamous cell carcinoma (80). The antibody inhibited proliferation of SSC-1 and FaDu cells up to 57%. Inhibition of collagen degradation was noted as well. Significant tumor growth inhibition was noted in SSC-1 xenografts. CNTO 3899 augments radiation response of SSC-1 and FaDu cells in vitro and in xenografts. Furthermore, the same study showed that CD147 function is associated with cytokine production of proinflammatory and proangiogenic factors such as IL1β, IL6, IL8 and VEGF. Inhibition of cytokines, MMPs and VEGF seems to mediate the mechanism of action of this mAb. The studies as outlined would suggest that inhibition of proliferation of tumor cells by CD147 mAbs in the absence of immune effector cells is either cell-type-specific and/or might be dependent on distinct epitopes the mAbs are directed against. Inhibition of T-cell activation and/or depletion was not investigated with CNTO 3899, Hb18G or Licartin. Efficacy data with staged xenografts (≥100 mm³) would be helpful to document the in vivo potency of therapeutic CD147 mAbs.

**CD147 as a Target for Treatment of Cancer**

Target validation experiments with mAbs and RNAi directed against CD147 have been summarized in the preceding sections of this review. Since the molecular interactions of CD147 with associated proteins are poorly defined, interference with small molecules is presently in the focus of drug development. The most obvious mode of intervention is to block CD147 function with mAbs. RNAi-mediated intervention would need more progress regarding the targeted delivery to cancer cells.

A critical issue is the broad expression of CD147, requiring toxicity studies for mAbs in a cross-reactive species. The function of CD147 as a mediator across the blood brain barrier would call for appropriate experiments for a therapeutic mAb. Expression of CD147 on hematopoietic cells and its function as a lymphocyte activation antigen are also critical issues. On the other hand, dependence of tumor cells on energy supply by anaerobic glycolysis (Warburg effect) and inhibition of the latter by CD147 mAbs which disrupt interaction with MCT and amino acid transporters makes tumor cells vulnerable to modulation of CD147 function. Antibody-dependent cellular cytotoxicity positive and negative mAbs should be evaluated preclinically to define the optimal format of the mAb. Unfortunately, the possible immunosuppressive effects caused by modulation of CD147 function cannot be modeled appropriately in in vivo efficacy models. The impact of CD147 modulation and inhibition of MMPs is a controversial issue since pro- and antitumoral activities are associated with MMPs and the consequences of CD147 modulation regarding tumor stroma interactions have to be dissected in more detail. However, CD147 clearly impacts on invasion, proliferation, angiogenesis, tumor cell metabolism such as glycolysis, and mediates prosurvival signals, multi-drug resistance and PI3K/Akt signaling, which are all hallmarks of oncogenesis.

**References**

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