Progress in Histochemistry and Cytochemistry 51 (2016) 25–32

Contents lists available at ScienceDirect

Progress in Histochemistry and Cytochemistry

journal homepage: www.elsevier.de/proghi

L1CAM: Cell adhesion and more

Timur R. Samatov[a,b,*], Daniel Wicklein[c], Alexander G. Tonevitsky[d,e,**]

[a] SRC Bioclinicum, Ugreshskaya str 2/85, 115088 Moscow, Russia
[b] Moscow State University of Mechanical Engineering, Bolshaya Semenovskaya str 38, 107023 Moscow, Russia
[c] Institute of Anatomy and Experimental Morphology and University Cancer Center Hamburg (UCCH), University Medical-Center Hamburg-Eppendorf, Martinistr. 52, 20246 Hamburg, Germany
[d] Hertsen Federal Medical Research Centre of the Ministry of Health of the Russian Federation, Koroleva str 4, 249036 Obninsk, Russia
[e] Moscow State University, Leninskie Gory, 119991 Moscow, Russia

ARTICLE INFO

Article history:
Received 4 April 2016
Accepted 20 May 2016

Keywords:
Cell adhesion
L1CAM
Ig-like domains
Cancer

ABSTRACT

L1CAM is a cell adhesion molecule of the immunoglobulin superfamily which was originally discovered as a major player in the development of the nervous system. L1CAM was demonstrated to have prognostic value in different cancers and to be a promising target for anti-cancer therapy. Here we overview the present data on L1CAM structure and function, regulation of its expression, role in cancer and therapeutic potential.

Contents

1. Introduction

Cell surface proteins mediating the interaction of cells with other cells or extracellular matrix are termed cell adhesion molecules (CAMs). They play essential roles in tissue morphogenesis and the maintenance of the multicellular structure of

* Corresponding author at: SRC Bioclinicum, Ugreshskaya str 2/85, 115088 Moscow. Russia.
** Corresponding author at: Hertsen Federal Medical Research Centre of the Ministry of Health of the Russian Federation, Koroleva str 4, 249036 Obninsk, Russia.
E-mail addresses: t.samatov@bioclinicum.com (T.R. Samatov), tonevitsky@mail.ru (A.G. Tonevitsky).

http://dx.doi.org/10.1016/j.proghi.2016.05.001
0079-6336/

living organisms. The CAMs are also involved in signal transduction and the development of various pathologies including infectious diseases and cancer [33]. There are five major classes of CAMs: cadherins, immunoglobulin superfamily proteins, selectins, mucins and integrins [56].

L1CAM is a CAM of the immunoglobulin superfamily which was originally discovered as major player in the development of the nervous system [61]. In later studies, it was reported to have various activities, in particular to be involved in malignant progression of neoplasia and the metastatic cascade [48]. Accordingly, L1CAM was demonstrated to have prognostic value in different cancers [1] and to be a promising target for anti-cancer therapy [11]. Here we review the present data on L1CAM structure and function, regulation of its expression, its role in cancer and its therapeutic potential.

2. L1CAM architecture and evolution

L1CAM is a CAM of the immunoglobulin superfamily which has initially been described as a cell surface antigen of the central nervous system [70]. The 200–220 kDa transmembrane glycoprotein consists of a conserved cytoplasmic part, five fibronectin type III repeats and six immunoglobulin-like (Ig) domains (Fig. 1) [61]. The domains Ig1-4 have four glycosylation sites, Ig5-6 have five and fibronectin domains have twelve [89].

Ig domains were originally described as an important part of antibodies, and genes encoding them exist in all metazoans including even sponges [78]. More complex metazoan genomes contain up to several hundred genes encoding Ig domain molecules, the majority being membrane or secreted proteins [3]. In many of these proteins, the Ig domains are combined with fibronectin type III domains. These facts confirm the hypothesis that L1-type genes arose early during metazoan evolution and evolved independently from other cell adhesion molecule gene families encoding proteins with different domain structures. More specifically, members of this gene family were found in different metazoan phyla, including Chordata,

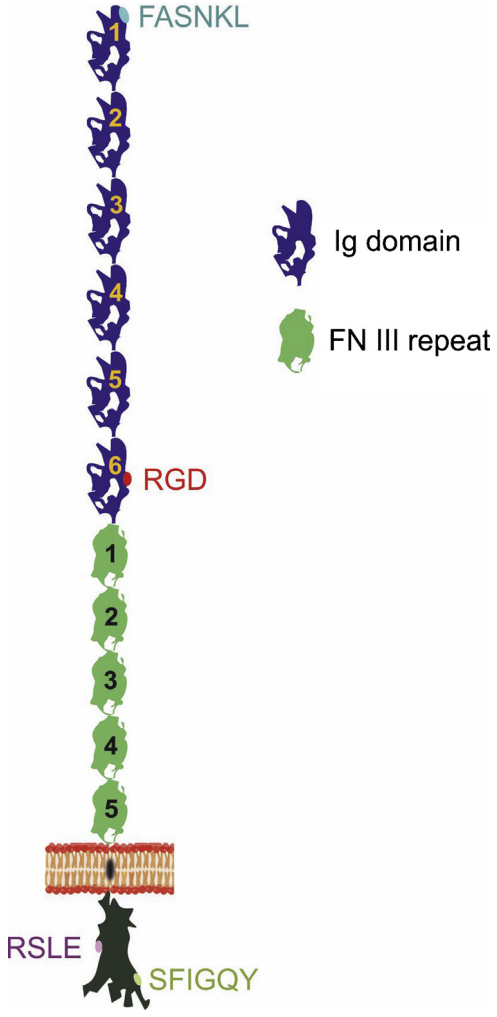

Fig. 1. Architecture and sequence motifs important for interactions of L1CAM.

Arthropoda, Echinodermata and Nematoda, and the origin of L1CAM goes back to the time in evolution when first primitive neural networks appeared [62].

The domain organization is common for all human L1 family members including L1-CAM (CD171), close homolog of L1-CAM (CHL1), neurofascin and NgCAM-related cell adhesion molecule (N-CAM) [38]. These protein variants are characteristic for all vertebrate L1 family genes whereas e.g. arthropod genomes were reported to have only one L1-type gene. This observation indicates that an ancestral single precursor L1-type gene of arthropods underwent two sequential genome duplications resulting in four homologous sets of genes in vertebrates [67]. For L1CAM, the human gene has 99% homology compared with the rhesus gene, 89% to rat, 88% to mouse, 49% to chicken, 41% to zebrafish and 30% to *Drosophila* genes, with the number and location of the Ig-like and fibronectin-like domains being conserved between the species [90]. Remarkably, the exon 27 coding for a C-terminal RSLE motif is a common feature for all vertebrates and is only missing in arthropod L1-type genes [39].

3. L1CAM interactions and functions

3.1. Ig-like domains

Ig-like domains are very efficient in recognizing and binding specific partners. In the various CAMs these domains exhibit mechanistic diversity because of the variable arrangements of these modules. Structural studies revealed a typical horseshoe structure formed by sequential Ig-like motifs [23]. The cooperative interactions of Ig-like domains are crucial for proper adhesion and functioning of L1CAM [36]. Their importance is confirmed by the fact that alcohol binding within a pocket bordered by Glu-33 and Tyr-418 amino acid residues inhibits cell adhesion by disrupting the Ig1-Ig4 interaction [18].

Furthermore, Ig-like domains play a major role in homophilic interactions between adjacent cells. Notably, this type of interaction is moderately conserved through evolution, and even only distantly related L1CAM proteins still exhibit a low binding affinity toward each other [41]. Co-immunoprecipitation studies of human truncated forms of L1 and endogenous full-length L1 showed that they interact homophilically *in trans* via the Ig1-4 domains (Fig. 1) [31]. The detailed kinetic studies using a surface plasmon resonance approach identified the K_D of the whole ectodomain – whole ectodomain interaction to be 116 ± 2 nM, and the K_D of the whole ectodomain – Ig1-4 interaction to be 130 ± 6 nM [31]. These data confirm that Ig1-4 domains are the main L1CAM part responsible for homophilic interaction. The cryo-electron tomography reconstruction of the interfaces between L1CAM-presenting liposomes suggested the horseshoe structure to be formed by these domains which is consistent with earlier crystallography data [37]. Additionally, it was shown that glycosylation modifications are involved in L1CAM homophilic interaction and that the whole protein can be considered as a sialic acid-binding lectin [50]. More specifically, the sites of Ig1-4 glycosylation being highly conserved in mammals are responsible for the distances between horseshoe dimers, thus modulating homophilic interaction patterns [89].

The Ig-like domains are also involved in a number of heterophilic interactions. The binding partners include Ig superfamily proteins NCAM, TAG-1/axonin, F11/contactin [69,66,62]. Binding of the highly glycosylated mucin type glycoprotein CD24 depends on its modification with α2,3-sialic acid, and this interaction occurs *in trans* between CD24 and L1CAM at the surface of neurite outgrowth-competent cells [50]. The FASNKL sequence in Ig1 domain is responsible for *cis* binding of neuropilin-1 (NP-1), a component of the semaphorin 3A (Sema3A) receptor complex [9]. The Ig1 domain is also involved in the interactions with proteoglycans neurocan and phosphacan [64,65], and the Ig domains are potential binding partners of extracellular matrix molecules laminin and tenascin [58].

An important group of proteins heterophilically interacting with L1CAM is the receptor tyrosine kinases. More specifically, during nervous system development Ig-like domains of L1CAM bind to EGFR [17], and this important regulatory event is evolutionary conserved from *Drosophila* to humans [51,63]. Interestingly, another tyrosine kinase receptor FGFR1 was shown to interact with L1CAM fibronectin domains (see below).

The sixth immunoglobulin-like domain of L1CAM contains an Arg-Gly-Asp sequence (RGD-motif) conserved between mammalian species [90]. This motif is responsible for integrin binding *in cis* [20,91]. L1CAM binds the integrin complexes with the following hierarchy: αVβ3 or αIIbβ3 > αVβ1 > α5β1 [20]. Interestingly, fibronectin domains are also involved in the interaction with integrins (see below). This type of interactions with integrins is also characteristic for the soluble shedded ectodomain of L1CAM, and they have been shown to be implicated in angiogenesis via VEGF receptor-2 tyrosine phosphorylation [24]. The recruitment of integrins is facilitated by a conformational change of the ectodomain leading to homomultimerization and interaction with the third fibronectin type III domain (see below). Binding with integrins initiates a signaling cascade activating focal adhesion kinase (FAK) which interacts with cytoplasmic tails of β1-, β2- and β3-integrin subunits. The activated FAK changes its conformation to active state and binds Src forming the active FAK/Src complex with both components being autophosphorylated [32]. This complex in turn activates downstream signaling pathways including PI3 K/Akt and mitogen-activated protein kinases (MAPK) [34].

3.2. Fibronectin domains

The L1CAM fibronectin type III (FN) modules I–V were shown to directly interact with the immunoglobulin modules of fibroblast growth factor receptor 1 (FGFR1) in an ATP-dependent manner [52] and this interaction plays an important role in modulating neuronal differentiation and FGFR1 phosphorylation.

The third FN-like repeat of L1 (FN3) spontaneously homomultimerizes forming trimeric and higher order complexes. These complexes are known to support direct RGD-independent interactions with several integrins, including αVβ3, α5β1 and α9β1 [84].

3.3. Intracellular part

Ankyrins are among the most important cytoplasmic binding partners of the intracellular section of L1CAM. L1CAM-ankyrin interaction is temporally and spatially tightly controlled and determines L1CAM function in different pathways [40]. The two sites for high-affinity binding of L1CAM are located within so-called "ank repeats" also known as membrane-binding domains [6]. Ankyrins bind to a 30-aminoacid sequence in the L1CAM cytoplasmic part containing the motif SFIGQY [25]. Remarkably, this sequence is highly evolutionary conserved and L1CAM-ankyrin interaction is observed even in arthopods [41]. It was shown that phosphorylation of the SFIGQY tyrosine residue completely abolishes ankyrin binding [30], and this is valid also for the Nrg protein of *Drosophila* [19]. Mutations of this sequence are implicated in the Gareis-Mason syndrome with severe neurological symptoms in males [74]. It was shown that L1CAM indirectly binds to the cytoskeletal component spectrin via ankyrin [8]. These data indicate that the connecting with the cytoskeleton is the important central aspect of L1CAM function.

A neuronal form of L1CAM containing exon 27 in its C-terminal part (and hence displaying the RSLE motif on protein level [47]) can bind Adaptor Protein 2 (AP2) which is involved in clathrin-mediated endocytosis and this binding is regulated by the phosphorylation of the tyrosine residue immediately upstream of the motif [75,90].

The same RSLE motif was demonstrated to be responsible for interaction with the FERM domain of a number of so-called ERM proteins including radixin, moesin and ezrin. This domain is highly conserved among ERM proteins and is responsible for membrane association by direct binding to the cytoplasmic domain of L1CAM. This binding is dependent on the phosphorylation of the upstream tyrosine [72]. Ezrin is a linker protein connecting L1CAM to actin [14]. Remarkably, the exon 27 and hence RSLE motif are missing in the Drosophila's L1-like protein Nrg, nonetheless, genetic interaction assays revealed the interaction of Nrg with ERM protein moesin [83].

Mouse model microtubule associated protein 2c (MAP2c) was identified as one more binding partner of the cytoplasmic L1CAM domain [68] providing one more link to the cytoskeleton.

These multiple binding events suggest that the cytoplasmic part of L1CAM can serve as a scaffold for the assembly of cytoskeleton components [54].

4. Regulation of L1CAM expression

Like the majority of neuron-specific genes, the L1CAM gene is regulated on the transcriptional level by RE1-Silencing Transcription factor (REST) [45]. This repressor is almost absent in neurons and certain cancer cells and highly abundant in all other cells. Another regulating L1CAM repressor is nuclear factor 1-A (NF-1A) [80]. The positive regulators of expression are transcription factor Slug/SNAI2 and T-cell factor activated by treatment with TGF-β1 and β-catenin signaling [26,28].

The L1CAM protein exists in two splicing isoforms. In neuronal and cancer cells the splicing factor Nova2 (neuro-oncological ventral antigen 2) provides for the expression of full-length L1CAM [60]. In all other cells Nova2 expression is repressed by high levels of REST and due to the expression of the splicing factor Nova2 two exons are removed from L1CAM mRNA, namely E2 from the N-terminal and E27 from the C-terminal domains. Exon E2 encodes a motif which is required for the optimal binding of L1CAM to neural ligands including the protein itself for homophilic binding, glycophosphatidylinositol-linked Ig superfamily members TAX-1 and F3, and hence is likely to be important for nervous system development [13]. The motif encoded by exon 27 was reported to be involved in clathrin-mediated endocytosis of L1CAM [90].

L1CAM was reported to be targeted by several miRNAs which are known to be rapidly regulated [98]. miR-146a down-regulates L1CAM and suppresses gastric cancer cell invasion and metastasis both *in vitro* and *in vivo* in human tumors xenografted into immunodeficient mice [42]. It was shown in endometrial carcinoma cells that another targeting L1CAM miRNA is miR-34a inhibiting cell migration. Remarkably, in patient primary tumor sections the inverse correlation of L1CAM and miR-34a expression was detected [79]. Similar results were obtained for miR-503 in osteosarcoma [10] and glioma [55]. Interestingly, miR-21-3p was reported to be a positive regulator of L1CAM expression [15]. Overexpression of miR-21-3p significantly increased L1CAM expression levels in renal, endometrial and ovarian carcinoma cell lines by transcriptional activation via an as yet unknown mechanism. The results were confirmed by a strong positive correlation between L1CAM and miR-21-3p expression levels in patient cohorts from renal, endometrial and ovarian cancers. Moreover, the combination of L1CAM and miR-21-3p as markers for overall and disease free patient survival improved the predictive power.

5. L1CAM and cancer

5.1. Role in EMT

Epithelial-mesenchymal transition (EMT) is a multi-step morphogenetic process during which static epithelial cells lose cell to cell junctions and apico-basal polarity and become migratory mesenchymal-like cells. This transition mimics the

Table 1
Prognostic value and therapeutic potential of L1CAM.

Cancer entity	Prognostic value	Drug/effect
Melanoma	Patients with L1-positive melanomas had a significantly higher risk for the development of metastases [87,99]	L1-11A antibody reduced migration and invasion of cells [59]
Breast cancer	High L1CAM expression was associated with nodal involvement, high grading, Her-2, negative ER-status, a shorter disease-free and overall survival [81]	shRNA-mediated knock-down inhibited migration and invasion of cells [92]
Head and neck cancer	High L1CAM expression was associated with metastasis and death in adenoid cystic carcinoma of the salivary glands [12]	shRNA-mediated knock-down in mouse xenograft model resulted in prolonged animal survival and complete suppression of tumor progression [43]
Pancreatic cancer	Positive L1-CAM expression was associated with node involvement, vascular and perineural invasion, higher degree of pain and with poor survival [5]	shRNA-mediated knockdown of L1CAM significantly inhibited cell proliferation and reduced the number of invasive cells [4]
Colorectal cancer	High L1CAM expression was associated with short survival and poor prognosis [7]	L1-11A and chCE7 monoclonal antibodies inhibited cell proliferation in vitro [94]
Ovarian cancer	L1CAM was found to be overexpressed in ovarian carcinoma and associated with short survival [22]	(177)Lu-modified antibody chCE7 was used for radioimmunotherapy of xenograft mice inhibited tumour growth and increased survival [21]
Non-small cell lung cancer	L1CAM was identified as a predictor of survival [86]	shRNA-mediated knock-down suppressed tumor growth and metastasis in xenograft model [35]
Neuroblastoma	L1CAM was identified as a malignancy biomarker [71]	In xenograft model shRNA targeting of L1CAM expression suppressed tumor growth and increased survival [2]
Prostate cancer	Expression of L1CAM correlated with prostate cancer metastasis [85]	In vivo targeting of L1CAM expression using liposome-encapsulated siRNAs effectively inhibited prostate cancer growth in mouse bone [85]

normal developmental process of gastrulation, in which cells from the epithelial sheet of the ectoderm start to form the third germinal layer, the mesoderm, whose migratory cells are called mesenchymal cells [46]. Oncogenic EMT recapitulates typical developmental features of the transition and plays a key role in metastases formation during malignant progression [73]. Various cell adhesion molecules and their glycosylation enzymes are involved in EMT contributing to changes in cell–cell and cell-matrix interactions resulting in a migratory and invasive phenotype [53].

The link of L1CAM and EMT was discovered in 2006 by Shtutman et al. The authors demonstrated that L1CAM expression resulted in the disruption of E-cadherin-mediated adherens junctions and increased β-catenin-dependent transcription in the human breast cancer cell line MCF7 leading to an increased motility of the cells. In addition, elevated levels of L1CAM were found *in vitro* in the leader cells of migrating monolayers which was consistent with the previously reported observation that this protein is highly abundant at the invasive front of tumors [26,22]. Remarkably, knock-down of L1CAM significantly decreased the cell motility in a wound healing assay and restoration of its expression completely restored the former cell phenotype [82]. These data indicate that L1CAM acts as a trigger for EMT in epithelial cells.

These results were further supported by the observations that treatment with TGF-β1 increased the expression level of the EMT initiating transcription factor Slug/SNAI2 which in turn up-regulated L1CAM in breast and pancreatic cancer cell lines [49,28], endometrial carcinoma [44] and non-small cell lung cancer cells [86]. The Slug-L1CAM regulatory axis has been reported to function in early stages of pancreatic and colon cancer progression [29,77] and development of chemoresistancy in pancreatic adenocarcinoma [57]. Chronic inflammation in inflammatory bowel disease patients activates this axis in inflamed intestinal epithelia and an elevated L1CAM expression level is associated with the presence of macrophages in tissue prone to malignant transformation [77]. Interestingly, L1CAM itself appears not to be an independent EMT driving force in colon cancer cell lines where it does not affect expression levels of E-cadherin [27].

Taken together, all these data confirm L1CAM to be an important player in the ensemble of EMT regulators extending its functionality far beyond simple mechanistic cell adhesion aspects.

5.2. Prognostic value and therapeutic potential

L1CAM was identified as a prognostic marker for a wide spectrum of malignancies including melanoma, neuroblastoma, pancreatic, prostate, ovarian, colorectal, breast, head and neck, non-small cell lung cancer (Table 1). The link of L1CAM with tumour aggressiveness and advanced cancer stages is consistent with its role in EMT. Interestingly, only in case of pediatric neuroblastoma L1CAM was found to predict good outcome in contrast to the adult tumours [88]. All these data in combination with the fact that antibodies or small-molecule ligands do not have to cross the membrane to bind cell surface L1CAM support the potential of this protein as a target for anti-cancer therapy.

The development of L1CAM-specific therapy is focused on two types of reduction of the activity of this protein, namely shRNA and antibody-based approaches (Table 1) [11]. The RNA interference-based approach employs siRNAs and shRNAs delivered by liposomes or lentiviral vectors shown to be efficient against aggressiveness of head and neck and prostate cancers [43,85]. In addition, neuroblastoma was reported to become more chemosensitive after targeting of L1CAM with siRNAs [93]. Besides, the efficacy of an shRNA approach was confirmed by in vitro assays and xenograft models for breast [92], pancreatic [4] and non-small cell lung cancer [35]. Another approach is based on anti-L1CAM antibodies. A successful

example is the monoclonal antibody L1-11A reported to have a dose-dependent inhibitory effect on tumour growth on xenografted ovarian cancer cells in mice [94]. Interestingly, similar treatment using L1-9.3/2a antibody affected local tumour growth in mice with syngeneic melanoma and pancreatic adenocarcinoma, and this treatment had no side effects and resulted in significant changes of the expression profile of cancer-related genes [16]. Combination of the antibodies with chemotherapeutic agents remarkably improved the response of pancreatic and ovarian carcinoma in a xenograft model [76]. L1CAM-specific antibodies are also efficiently used for radioimmunotherapeutic approaches when labeled with low energy β-emitters 177Lu and 67/64Cu [95]. In more detail, a mouse xenograft model of disseminated ovarian cancer showed significant retardation of tumor growth and prolonged survival when the 177Lu-labeled anti-proliferative antibody chCE7 was used. Notably, the biodistribution analysis demonstrated high and specific accumulation of radioactivity at the tumor site [21]. This antibody was mutated to achieve more rapid blood clearance, labeled with 67Cu and demonstrated to significantly reduce tumor growth and prolong survival in mouse xenograft model of ovarian cancer [97]. Using a similar ovarian cancer model 161Tb label was reported to inhibit tumour growth more efficiently than 177Lu [96].

6. Conclusions

L1CAM is a neuronal cell adhesion molecule involved in the development of the nervous system and progression of malignancies. The detailed mechanisms of regulation of its expression in different tumours are still to be elucidated, but the reported prognostic value in a wide spectrum of cancers proves the importance of this protein as a biomarker. Besides, the growing body of data suggests that targeting L1CAM can be the basis for efficient anticancer therapy. These facts highlight the potential of L1CAM and indicate that it will stay in the focus of fundamental and biomedical research within the next years.

Acknowledgement

This work was supported by the Russian Scientific Foundation (Grant 14-44-00051).

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