Neural cell recognition molecule L1: relating biological complexity to human disease mutations

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Human single gene disorders that affect the nervous system provide a host of natural mutations that can be deployed in the quest to understand its development and function. A paradigm for this approach is the study of disorders caused by mutations in the gene for the neural cell recognition molecule L1. L1 is the founder member of a subfamily of cell adhesion molecules that are primarily expressed in the nervous system, and to date it is the only one to be associated with a hereditary disease. In this review we will summarize how the analysis of pathological mutations in L1 is complementing the study of mouse models and in vitro analysis of L1 function.

INTRODUCTION

L1 is one member of a subfamily of four mammalian cell surface glycoproteins that are related by structure and sequence (Fig. 1). They belong to the large class of immunoglobulin superfamily cell adhesion molecules (CAMs) that can mediate cell–cell adhesion through Ca²⁺-independent homo- or heterophilic binding at the cell surface (1,2). Each member of the L1 subfamily contains six Ig-like domains linked to five fibronectin type III (FNIII) domains on the extracellular surface, a single-pass transmembrane domain and a short, but highly conserved, cytoplasmic tail. L1 or L1-like molecules with this structure have been identified in many species including rodent, fish, fruit fly and nematode. In each case they have proven to be important components of the ligand–receptor network of guidance forces that influence axonal growth and guidance (for reviews see refs 3–5). For this reason L1 is more aptly called a neural recognition molecule rather than adhesion molecule.

In mammals, L1 is expressed throughout the nervous system on subsets of developing and differentiated neurons as well as on Schwann cells of the peripheral nervous system. On differentiated neurons L1 is found at regions of contact between neighbouring axons and on the growth cones, the structures at the leading tip of axons that are responsible for sensing extracellular growth and guidance cues. This distribution supports the suggestion from in vitro studies that L1 adhesive interactions may mediate axon bundling (fasciculation) (6,7) and that L1 acts as a growth cone receptor for signals that induce the extension of processes (neurites) from neurons in culture (8). Neurite outgrowth is likened to axon growth and is taken as evidence that L1 may be involved in axon growth during development. L1 is also involved in interactions between Schwann cells of the peripheral nervous system and axons (9,10), in neuronal cell migration (11), synaptogenesis, myelination, neuronal cell survival (12) and even long-term potentiation (13). Although primarily expressed in the nervous system L1 is also found at other specialized sites including a subclass of leukocytes, intestinal crypt cells and kidney tubule epithelia (14–16, and reviewed in ref. 17). As the human disorder involving L1 is essentially neurological we will focus here on its role in the nervous system.

MUTATIONS IN L1 AFFECT DEVELOPMENT OF THE NERVOUS SYSTEM IN MAN AND MOUSE

Mutations in the L1 gene are responsible for an X-linked recessive neurological disorder that has been described as X-linked hydrocephalus, MASA syndrome or spastic paraplegia type I (SPG1). The varying nomenclature is a reflection of extremely variable presentation both within and between families (reviewed in refs 18–20). The most consistent features in affected boys are degrees of lower limb spasticity, mental retardation, hydrocephalus and flexion deformity of the thumbs. Those that develop hydrocephalus (water on the brain) in utero or soon after birth have a lower life expectancy and many die neonatally. The excess of cerebrospinal fluid (CSF) and increased size of cerebral ventricles may partly result from stenosis of a thin channel called the aqueduct of Sylvius through which CSF circulates, although inadequate cell migration or loss of neurons may also contribute to the increase in cavity size. Perhaps the most striking pathological observation is hypoplasia or absence of two long axonal tracts, the corticospinal tract and the corpus callosum. The former is involved in the control of voluntary motor function and its disruption provides an explanation for the spasticity observed in the patients. The corpus callosum is the large bundle of nerve fibres that connects the two cerebral hemispheres and its underdevelopment may contribute to the mental retardation or epilepsy observed in patients with abnormal L1. Other frequent

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The mechanism by which L1 mutation disrupts the development of the CST in knockout lines involves failure in axonal pathfinding at a time when these axons are required to grow across the midline (25). Presumably, L1 is required as an attractive or repulsive cue, substrate or neuronal receptor at this point in axon guidance. The underdeveloped CST observed in mouse and man could result from degeneration of neurons that do not synapse with the correct targets. In keeping with the extreme interfacial as well as intrafamilial variability seen for the condition in man, some aspects of the knockout phenotype in mouse vary markedly on different genetic backgrounds (24), indicating the epistatic influence of other genes on the ‘penetrance’ of the L1 mutation.

Overall, the phenotypes observed in mouse and man show that L1 function is clearly pivotal for generation of certain axonal tracts and important for correct cortical and cerebellar morphology.

**HOW DOES L1 FUNCTION?**

It is apparent that L1 participates in a variety of different cellular responses both within and outside the nervous system. This flexibility of function is probably related to a multiplicity of binding partners, potential signalling routes and post-translational modifications.

**Cell surface ligands**

A bewildering array of molecules have been found to bind to the extracellular domains (ECDs) of L1. These include L1 itself, other members of the Ig superfamily, integrins, extracellular matrix proteins such as laminin and a variety of proteoglycans (reviewed in refs 17, 26, 27) (Fig. 2). Where and when these different interactions are relevant is now the challenging question. Homophilic binding, i.e. L1 acting as ligand and receptor, will promote both cell adhesion and neurite outgrowth from explanted neurons, indicating that this interaction may be relevant to both fasciculation and axon growth. Furthermore, cerebellar neurons from L1 knockout mice will no longer extend processes on purified L1 (22). However, heterophilic ligands for L1 may also influence L1 activity. In several cases this influence is exerted in the plane of the neuronal membrane (in cis). For example, L1 binds in cis to TAG-1 a member of another subfamily of Ig CAMs (28) and this interaction may even be required for L1–L1 mediated neurite outgrowth from specific neurons (29, 30). DM-GRASP, another cell surface adhesion molecule, may also cooperate with L1 to promote neurite outgrowth (31). Cis interaction with NCAM has been shown to enhance the ability of L1 to bind homophilically (32) and the small cell surface glycoprotein nectadrin (CD24) may also be recruited to this complex (33).

Not all of L1’s potential interactions result in a positive effect on adhesion or axon growth. For example, L1 binds with strong affinity to neurocan, a neural, extracellular matrix chondroitin sulfate proteoglycan that has an inhibitory effect on both neuronal adhesion and neurite outgrowth (34).

**Signalling**

Influences on cell behaviour imply that L1 ligand binding must be linked to intracellular signalling pathways. So how does a
cell surface glycoprotein with no self-contained catalytic domains engage a signalling pathway? One obvious way is by binding to molecules that can themselves elicit signal transduction. At least one mechanism through which L1–L1 recognition can stimulate axonal growth is by activating the tyrosine kinase-linked receptors for fibroblast growth factors (FGFRs). The downstream signalling cascade involved has been worked out in considerable detail and culminates in transient influx of calcium through L- and N-type channels at localized sites in the growth cone (35,36). Growth cone morphology and behaviour are known to be sensitive to waves of calcium with increased concentration generally slowing neuritic growth (37,38). How focal domains of calcium conversely promote growth cone advance is not understood, although one possibility is through the action of Ca^{2+}-dependent modifiers of the actin cytoskeleton.

Dimerization with TAG-1 offers yet another mechanism through which L1 may influence intracellular events as the chick homologue of TAG-1 (axonin-1) is associated, through an unknown linker, with non-receptor tyrosine kinase (39). Components of the MAPK (mitogen-activated protein kinase) signalling pathway (40), tyrosine kinase src and pertussis toxin sensitive G proteins have also been implicated in L1 mediated responses (41,42).

**INTERACTIONS OF THE CYTOPLASMIC DOMAIN**

The 100 or so amino acid stretch of the cytoplasmic domain is the most conserved region between L1-like molecules in all species and its functional importance is highlighted by the finding that deletions or even single amino acid changes in this region can cause neurological disease in man. Members of the L1 subfamily share an amino acid sequence that has high affinity for ankyrin, a linker protein of the spectrin-based cytoskeleton that underlies the plasma membrane (43,44). Ankyrins are multi-domain proteins that can bind both cytoskeletal and integral membrane components and two forms (ankyrinB and ankyrinG) are abundant in the nervous system (45). Mobilization of ankyrin to sites of cell contact is observed on homophilic binding of L1 or on formation of a TAG-1–L1 complex (28). Thus CAM adhesion is translated into mobilization of cytoskeletal components and potentially of other integral membrane proteins. The ability of L1-like molecules to bind ankyrin is regulated by phosphorylation of a highly conserved tyrosine residue within the L1 cytoplasmic motif FIGQY (46). Interestingly, deletion, mutation or phosphorylation of this motif has an inside-out effect on the ability of L1-like CAMs to bind homophilically (46), suggesting that ankyrin recruitment may have a role in stabilization of intercellular contacts that involve L1. A role for ankyrin in L1 function is supported by the remarkable overlap between the phenotype of ankyrinB−/− mice, L1-deficient mice and human subjects with L1 mutations (47). The cytoplasmic domain can also associate with actin stress fibres (48).

The cytoplasmic domain also houses a tyrosine-based sorting motif, YRSL (or more generally Yxxφ, where φ is an amino acid with a bulky hydrophobic side chain), that is required for the correct trafficking of L1 along axons to the...
growth cone as well as for L1 endocytosis (49,50, and see below). The YRSL motif binds to the adaptor complex AP-2 in a pathway of internalization via clathrin-coated pits.

**L1 modification**

L1 is subject to glycosylation and phosphorylation, both of which may affect its function. Modulation of L1 function by NCAM, for example, occurs through recognition of L1 carbohydrate (32). In addition to tyrosine phosphorylation of the ankyrin-binding domain, serine phosphorylation by specific kinases occurs at several sites (40,51–53). Interestingly, these sites are next to known binding domains for either cytoskeletal components or the AP-2 complex (Fig. 3A), suggesting that serine phosphorylation may influence cytoplasmic interactions, L1 mobility and internalization. Indeed, phosphorylation by ERK2 is found to be dependent on L1 endocytosis in vitro (40). ERK2 and p90rsk are components of the MAPK pathway, a signalling cascade that can also be activated by FGFRs. The possibility of cooperation between these pathways in modulating L1 action is intriguing.

It has been known for some time that in addition to full-length 200 kDa protein, fragments of L1 polypeptide can be found in tissue extracts, but it has been a matter of debate whether L1 is specifically cleaved. Recently, however, a
matrix metalloprotease (TACE) and plasmin have been shown to release L1 from the cell surface through cleavage at specific sites (54,55) (Fig. 3B). This raises the interesting possibility that as well as acting as a cell surface receptor, L1 may be released in a soluble form that could act as a ligand at some distance from its source. Along with regulated endocytosis this may modulate the spatial availability of L1 in response to intracellular signals. Such regulation may be very important, for example, at the growth cone where polarity of L1-mediated effects may influence axonal turning.

**L1 expression**

Further modification of L1 activity and localization is afforded by the use of specific splice variants. The L1 gene comprises 29 exons of which 28 are coding (56,57). Studies on its transcriptional regulation have revealed that its neural expression is at least partly determined by a neuron restrictive silencing element (NRSE) contained within the second intron and a homeobox- and paired-domain-containing region (HPD) in the first intron (57,58). Neural expression is also associated with inclusion of two mini exons (exons 2 and 27) that are excluded from the non-neural transcript (59,60). Exon 27 (encoding RSLE) has a neuron-specific function as it contributes to the YRS motif that is required for targeting to the growth cone. As this sequence is lacking outside the nervous system AP-2-mediated endocytosis of L1 is presumably also restricted to neural tissue. The function of exon 2 is unknown although preliminary results indicate that it may modulate homophilic and heterophilic ligand binding (E. De Angelis, unpublished data).

**MUTATIONS IN L1: INFERENCE FOR L1 FUNCTION**

Since our first description of an L1 mutation (61), 91 different mutations have been identified in >100 families (62, and references therein). Most forms of mutation have been reported and they are distributed across all domains (38 missense, 10 nonsense, 21 frameshift, 17 splicing, 5 intragenic deletion and 1 intragenic duplication). Most of the nonsense and frameshift mutations would eliminate cell surface expression of L1 intragenic duplication). Most of the nonsense and frameshift mutations would eliminate cell surface expression of L1 through truncation prior to the transmembrane domain. Although the mutations are heterogeneous, attempts to relate position and type of disruption to phenotype are confounded by intrafamilial variability in presentation. Nevertheless, mutations of the cytoplasmic domain are less likely to cause life-threatening hydrocephalus than those disrupting the extracellular surface, indicating some qualitative difference in the effect of these two broad classes of mutation (63). As homophilic binding may be preserved in the absence of the cytoplasmic domain (64,65) the relative longevity of patients with cytoplasmic mutations may be due to preservation of some of L1’s ‘adhesive’ functions.

**CYTOPLASMIC DOMAIN**

Five mutations truncate the cytoplasmic domain at various points along its length and these can be related to known sites of L1 binding, modification or intracellular trafficking (Fig. 3A). Whereas some delete additional functional sites, all eliminate at least part of the conserved ankyrin-binding domain as well as the tyrosine residue at position 1229 that is involved in moderating ankyrin binding for the L1 subfamily (46,66). Mutation of Y1229 to histidine in one family confirms the importance of controlled ankyrin binding for L1 function in the nervous system. No mutations have been found to date that mutate serines phosphorylated by p90rsk (S1152), casein kinase II (S1181) or ERK2 (S1204 and S1248). However, mutation of an additional serine at 1194 implicates this residue as a potential substrate for modification. Clearly mutations that delete the RSLE will also disrupt trafficking of L1 in differentiated neurons, only allowing transport of protein to the cell soma.

**MISSENSE MUTATIONS AFFECTING THE ECDS**

The most interesting mutations to consider with respect to gene function are the missense mutations and for L1 these represent the largest single class. The majority are distributed across 10 of 11 of the ECDs (Fig. 3B and Table 1). Outline structures for the ECDs based on the crystallographic resolution of related proteins allow predictions to be made concerning the structural outcome of many of these changes (67). Over half of the mutations that can be mapped onto these structures are found to affect residues that are key to the structural integrity of individual domains (Table 1). A smaller proportion affect residues that are not part of the core structure and are more likely to affect surface properties of L1 than domain integrity. Additional insight comes from the structural resolution of hemolin, an insect protein composed of four Ig domains with similarity to the first four domains of L1. In hemolin these domains are folded into a horseshoe structure through interaction between Ig domains 1 and 4 and between domains 2 and 3 (68). Interestingly, 50% of the human mutations affected Ig domains 1–4 lie in the contact regions that are required for this four-domain conglomerate.

Missense mutations can theoretically have a number of consequences for L1 production and function. Firstly, they may alter the folding or intracellular trafficking of L1. A mutation of the signal peptide (W9S), for example, has predictably been found to affect cell surface expression (69). Secondly, mutations that put a cysteine on the surface of the molecule, such as Y194C and Y1070C, could be affecting protein function or mobility by promoting inappropriate intermolecular disulfides either during folding or on the cell surface. Thirdly, and most intriguingly, some of these mutations may affect ligand binding and therefore offer the potential for mutation-based structure–function studies. Through analysing 12 mutations we have shown that missense changes in the ECDs have variable effects on binding of L1 to itself or to the related CAMs TAG-1/axonin-1 and F3/F11 (69) (Table 1). Whereas many mutations affect more than one binding activity, several have no influence or affect homo- or heterophilic binding selectively. As the latter are usually surface changes they may highlight key binding sites for specific ligands. For example, E309K is a surface mutation that does not affect homophilic binding but severely compromises binding to axonin-1 and F11. Interestingly, mutations affect TAG-1/axonin-1 and F3/ F11 binding in a similar way indicating that these two structurally related ligands interact with L1 in a very similar fashion. These studies indicate, for the first time, that pathogenesis may result from different effects on L1 ligand binding. That several
domains are required for correct homo- or heterophilic binding of L1 is also apparent. This is in contrast to results of a previous study using isolated domains, which suggested that only Ig domain 2 was required for homophilic interaction (70). This difference may be reconciled if Ig domain 2 contains an essential binding site but interaction requires considerable antiparallel overlap of L1 monomers. Figure 3B also shows the distribution of missense mutations alongside known binding regions for several L1 ligands (7,69–72). It is clear that domains of interaction overlap considerably and that human mutations may also be affecting binding to integrins or proteoglycans. Further studies on the effects of L1 mutations both on ligand binding and on intracellular processing will allow a much better understanding of disease pathology and the functional requirements of L1 domains, and will influence the design of animal models that test the relevance of disrupting specific aspects of L1 interaction and mobility.

SUMMARY

L1 is part of a dynamic complex of interacting molecules, associated signal transduction pathways and cytoskeletal connections. How these different pathways cross-talk to influence neurite outgrowth, stabilize cell adhesion or affect neuronal migration is unclear although some general principles do emerge. Firstly, L1 is not merely an intercellular glue but a receptor capable of transducing signals to the inside of the cell as well as responding to signals from the inside. Secondly, it has an intimate and plastic relationship with the cytoskeleton. Thirdly, L1 activity is mediated by a number of different binding partners, both in trans and in cis, as well as post-translational modification and cleavage. Mutation of L1 in man and mouse results in developmental defects that are consistent with a role for L1 in axonal pathfinding as well as cell migration. Assessment of the effects of disease-causing mutations on L1 function together with analysis of animal models is proving a valuable tool in relating L1 function to in vivo activity.

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REFERENCES


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