Differential Effects of Two Hydrocephalus/MASA Syndrome-related Mutations on the Homophilic Binding and Neuritogenic Activities of the Cell Adhesion Molecule L1*

Xiaoning Zhao and Chi-Hung Siu§
From the Banting and Best Department of Medical Research and Department of Biochemistry, University of Toronto, Toronto, Ontario M5G 1L6, Canada

The cell adhesion molecule L1 plays an important role in neural development. We have previously demonstrated that the second immunoglobulin-like domain (Ig2) of L1 contains both homophilic binding and neuritogenic activities (Zhao, X., and Siu, C.-H. (1995) J. Biol. Chem. 270, 29413-29421). Recently, two mutations (R184Q and H210Q) within the Ig2 region of the human L1 gene have been shown to be responsible for X-linked hydrocephalus and the related MASA (mental retardation, aphasia, shuffling gait, and adducted thumbs) syndrome. Glutathione S-transferase-Ig2 fusion proteins containing these mutations were used to evaluate their effects on L1. The homophilic binding activity of fusion proteins and their ability to promote neurite outgrowth from retinal cells were examined. The R184Q mutation led to a complete loss of both homophilic binding and neuritogenic activities, while the H210Q mutation resulted only in a partial loss. These results provide, for the first time, direct demonstration of the deleterious effects of hydrocephalus/MASA mutations on two intrinsic properties of L1.

The cell adhesion molecule L1 is expressed primarily in postmitotic neurons and has been implicated in neural migration, neurite outgrowth, and fasciculation during brain development (for a review, see Ref. 1). L1 is a 200-kDa transmembrane glycoprotein and a member of the immunoglobulin (Ig) superfamily of cell adhesion molecules. It contains six Ig-like domains in the amino-terminal region, followed by five fibronectin type III repeats, one transmembrane domain, and a cytoplasmic domain (2, 3). L1 can undergo homophilic interactions with L1 (4, 5), as well as heterophilic interactions with other adhesion molecules, such as NCAM (6), TAG-1/axonin-1 (7, 8), F3/F11 (9), glia (10), and components of the extracellular matrix (11, 12). In addition to cell adhesion, substrate-coated L1 is a potent inducer of neurite outgrowth from primary neurons (4, 5).

The human L1 cDNA has been cloned (13), and the gene has been mapped to chromosome Xq28 (14). Several recent reports show that a group of heterogeneous mutations in L1 are responsible for X-linked hydrocephalus and two related neurological disorders, MASA (mental retardation, aphasia, shuffling gait, and adducted thumbs) syndrome, and spastic paraplegia type 1 (15-17). Most of them are missense mutations, resulting in amino acid changes in the extracellular and cytoplasmic domains, while others are nonsense, deletion, or splicing mutations resulting in the truncation or secretion of L1. However, little is known about how these mutations give rise to these related neurological diseases. An investigation of the role of the mutated residues in L1 function is, therefore, crucial to our understanding of these defects.

We recently demonstrated that the Ig2 domain of L1 harbors both homophilic binding and neuritogenic activities (18). Interestingly, two missense mutations have been localized to Ig2. One results in the replacement of Arg-184 with Glu. This mutation is found in patients with severe hydrocephalus, which is characterized by the absence of the corticospinal tract and stenosis of the aqueduct of sylvius (16). The other mutation results in the substitution of His-210 with Glu and is detected in MASA patients with a milder phenotype (16). Those who survived suffered from mental retardation. We have investigated the effects of these two mutations on the homophilic binding and neuritogenic activities associated with L1 Ig2. These activities are completely lost in the R184Q mutation, but are only partially affected in the H210Q mutation.

EXPERIMENTAL PROCEDURES

Construction and Expression of Mutated Forms of L1 Ig-like Domain 2—The human L1 cDNA fragment coding for Ig2 was obtained by polymerase chain reaction amplification using the forward primer 5'-CGGGATCTCTGGCCAGGTTGCAGCCC-3' and the reverse primer 5'-GTGGATCCGACCCGGAGGTCAATG-3'. The polymerase chain reaction product was digested with BamHI and then subcloned into pBlueScript SKII(+). The oligonucleotides 5'-GTGACGA-3' and 5'-TCTGCCAGGCCCACTT-3' were used as mutagenic primers for Ig2mtl (R184Q) and Ig2mt2 (H210Q), respectively. The oligonucleotide 5'-ATAAGCTTGATCGCGAATTCCTGC-3' was used as the selection primer. Mutations were confirmed by double-stranded DNA sequencing. The inserts were then released from pBlueScript and subcloned into the pGEX-3T vector. Expression and refolding of the two mutant GST-Ig2 fusion proteins were carried out as described previously (18).

Cell Transfection—The full-length L1 cDNA (16) was subcloned into the unique HindIII site of the expression vector pRc/CMV (Invitrogen). An antisense-L1 construct was made by inserting the L1 cDNA in the reverse orientation. The Chinese hamster ovary cell line LR73 (19) was cultured in α-MEM containing 10% fetal calf serum until subconfluence. Cells were collected and plated on 10-cm dishes (at 1:10 dilution) 1 day before transfection. LR73 cells were transfected with 20 μg of plasmid DNA using the calcium phosphate precipitation method. Transfected clones were selected using 400 μg/ml G418, followed by limiting dilution and clonal analysis for L1 expression.

© 1996 by The American Society for Biochemistry and Molecular Biology, Inc.

*This work was supported by Operating Grant MT-11443 from the Medical Research Council of Canada. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§To whom correspondence should be addressed: Charles H. Best Inst., University of Toronto, 112 College St., Toronto, Ontario M5G 1L6, Canada. Tel.: 416-978-8766; Fax: 416-978-8528; E-mail: chi.hung.siu@utoronto.ca.

1 The abbreviations used are: NCAM, neural cell adhesion molecule; MASA, mental retardation, aphasia, shuffling gait, and adducted thumbs; Ig2, immunoglobulin-like domain 2; MEM, minimum Eagle's medium; GST, glutathione S-transferase.
Effects of Hydrocephalus Mutations on L1

RESULTS AND DISCUSSION

To investigate the effects of R184Q and H210Q mutations on the homophilic binding and neuritogenic activities of L1, Ig2 containing these mutations were expressed in bacteria as GST fusion proteins. GST-Ig2mt1 contained the R184Q mutation, while GST-Ig2mt2 contained the H210Q mutation. Purified proteins were analyzed by gel electrophoresis (Fig. 1). Under reducing conditions, both wild-type and mutant GST-Ig2 fusion proteins migrated with an apparent size of 100 kDa, as expected.

Binding of L1-conjugated Covaspheres to Cells—Rat L1 or recombinant GST-Ig2 was conjugated to Covaspheres as described previously (18). To assay for the binding of L1 Covaspheres to cells, L1-transfected LR73 cells were seeded sparsely on coverslips 20 h before the assay. Coverslips were blocked with 10% SDS-polycrylamide gel and stained with Coomassie Blue. Lane a, GST-Ig2; lane b, GST-Ig2mt1; lane c, GST-Ig2mt2; lane d, GST.

To directly test the homophilic binding activity of the mutant fusion proteins, binding of fusion protein-conjugated Covaspheres to substrate-coated protein was carried out. All fusion proteins adsorbed to Petri dishes with similar efficiency. As a
GST-Ig2mt1 substrate was observed (Fig. 3). However, GST-Ig2mt2 was reduced by GST-Ig2. However, binding of GST-Ig2 Covaspheres to GST-conjugated Covaspheres attached very well to substrate-coated GST was used as a negative control. GST-Ig2-adsorbed onto a Petri dish for Covasphere binding, while substrate-coated GST-Ig2mt1 only at background level (Fig. 3A), indicating that the Arg to Gln substitution led to the loss of its homophilic binding activity. In contrast, GST-Ig2mt2 still retained significant homophilic binding activity (Fig. 3C). Thus, the H210Q mutation had a milder effect on the homophilic binding activity of Ig2.

Next, the effects of these fusion proteins on neurite outgrowth were examined. The mean neurite length of neural retinal cells cultured on L1-expressing LR73 cells was ~3 times longer than those cultured on the antisense transfectants (Fig. 4A). When individual fusion proteins were included in this assay, GST-Ig2, being a strong competitor for L1 binding, reduced the mean neurite length to near background level, while GST-Ig2mt2 was reduced by ~50%, but no significant binding to the GST-Ig2mt1 substrate was observed (Fig. 3A). In addition, GST-Ig2mt1-conjugated Covaspheres attached to substrate-coated GST-Ig2mt1 only at background level (Fig. 3B), indicating that the Arg to Gin substitution led to the loss of its homophilic binding activity. In contrast, GST-Ig2mt2 still retained significant homophilic binding activity (Fig. 3C). Thus, the H210Q mutation had a milder effect on the homophilic binding activity of Ig2.

We have previously demonstrated that the L1 Ig2 fragment can serve as a potent substrate for neurite outgrowth from retinal cells (18). The effects of the two hydrocephalus/MASA syndrome-related mutations on the neuritogenic activity of Ig2 were examined. In comparison with cells cultured on the GST substrate, GST-Ig2 stimulated a 2.8-fold increase in the mean neurite length of retinal cells (Fig. 5). In contrast, GST-Ig2mt1 failed to promote neurite outgrowth from these cells. GST-Ig2mt2, on the other hand, retained substantial neuritogenic activity, and only a 20% reduction in the mean neurite length was observed.

The above results, taken together, demonstrate that the R184Q mutation abolishes both the homophilic binding activity and the neuritogenic activity associated with the Ig2 domain of L1, whereas the H210Q mutation results only in a partial loss of these two activities. It is likely that Arg-184 plays a crucial role in the structure and function of L1. Arg-184 is highly conserved in L1 among different species, including mouse (2), rat (3), chicken (20), and the Drosophila homolog (21). In addition, this residue lies within a region corresponding to the predicted C’ β-strand of the Ig fold, suggesting that the C’ region of L1-Ig2 may participate directly in L1-L1 homophilic binding. It is conceivable that the forward binding reaction may depend on electrostatic interactions involving Arg-184. Interactions centered at this region may then lead to other interactions at secondary sites along the length of the extra-cellular segments of two apposing molecules, further stabilizing the binding reaction. This possibility has been observed in NCAM, which is also a member of the Ig superfamily. NCAM homophilic interaction is centered around the predicted C’ β-strand and the C’ E loop in the third Ig-like domain (22). This region of the NCAM molecule is capable of undergoing isologous interactions with the same region of an apposing molecule (23). The charged residues in this region also appear to play a crucial role (22).

In both L1 and NCAM, homophilic binding is closely coupled to their ability to induce neurite outgrowth from neuronal cells (18, 24). Although several other structural domains in L1 have been implicated in neurite outgrowth promotion (25, 26), the severity of the neuropathological phenotype of patients with the R184Q mutation attests to the importance of the homophilic binding and neuritogenic activities centered around Arg-184. It is conceivable that homophilic binding may generate neurite outgrowth signals by inducing conformational...
Changes in the molecule and alter its interactions with other membrane or cytoplasmic components. Potential candidates involved in downstream events of L1-dependent neurite outgrowth include fibroblast growth factor receptor (27), pp60c-src (28), and ankyrin (29).

His-210 is predicted to lie within the \( \beta \)-strand of the Ig fold, with its charged side chain pointing outward on the surface of the molecule. Substitutions with another charged or polar residue are likely to be tolerated. Indeed, this residue is less conserved in L1 homologs, and His-210 is replaced by Asn in mouse and rat (2, 3) and by Ser in the Drosophila homolog (21). This is also supported by the less deleterious effects of the H210Q mutation. Therefore, a close correspondence exists between the in vitro activities of the mutant proteins and the pathological phenotype caused by these two mutations. Exactly how these two mutations affect neural development is not known. Further studies will depend on the availability of transgenic animals that express these mutant forms of L1.

To date, 23 mutations in L1 have been reported in hydrocephalus, MASA, and spastic paraplegia type 1 patients. These mutations are evenly distributed along the L1 molecule (1, 17), indicating that these structural domains may have important biological functions. Alternatively, some of the amino acid substitutions may induce conformational changes, causing the inactivation of functional domains at a distance. Mutations outside the Ig2 domain may affect heterophilic interactions of L1 with other matrix and membrane components. Recently, the RGD sequence located in the sixth Ig-like domain of L1 has been found to interact with the \( \alpha_5 \beta_3 \) integrin (30), and \( \alpha_5 \) is present predominantly in the glia of the central nervous system (31), suggesting a role for the L1 RGD sequence in neuron-glial interaction in the brain cortex and in the cerebellum. L1 is also known to interact heterophilically with axonin-1/TAG-1, F3/F11, and brain proteoglycans. It is evident that L1 has a very complex biology, and mutations affecting its interactions with different binding partners may have a wide range of effects on neuron migration and neurite outgrowth. Our future understanding of the diverse biological roles of L1 in brain development will depend on the identification of its binding sequences and the elucidation of their mechanisms of interaction.

Acknowledgments—We thank Dr. Vance Lemmon of Case Western Reserve University for the human L1 cDNA clone, Dr. Clifford Stanners of McGill University for the Chinese hamster ovary cell line LR73, and Drs. Andus Bennick and John Roder for advice and discussion.

REFERENCES

1. Wong, E. V., Kenwrick, S., Willems, P., and Lemmon, V. (1995) Trends Neurosci. 18, 168–172
2. Moos, M., Tacke, R., Schacher, H., Teplow, D., Friih, K., and Schachner, M. (1988) Nature 334, 701–705
3. Prince, J. T., Albert, L., Healy, P. A., Nauman, S. J., and Stallcup, W. B. (1991) J. Neurosci. Res. 30, 567–581
4. Lemmon, V., Farr, K. L., and Lagenaur, C. (1989) Neuron 2, 1597–1603
5. Miura, M., Asou, H., Kobayashi, M., and Uyemura, K. (1992) J. Biol. Chem. 267, 10752–10758
6. Kadmian, G., Kowitz, A., Altevogt, P., and Schachner, M. (1990) J. Cell Biol. 110, 193–208
7. Kuhn, T. B., Stockili, E. T., Condrau, M. A., Rathjen, F. G., and Sonderegg, P. (1991) J. Cell Biol. 115, 1113–1126
8. Felsenfeld, D. P., Hynes, M. A., Sklar, M., Furley, A. J., and Jessel, T. M. (1994) Neuron 12, 675–690
9. Brümmendorf, T., Hubert, M., Treubert, U., Lauschner, R., Tarnok, A., and Rathjen, F. G. (1993) Neuron 10, 711–727
10. Grumet, M., and Edelman, G. M. (1988) J. Cell Biol. 106, 487–503
11. Grumet, M., Friedlander, D. R., and Edelman, G. M. (1993) Cell Adh. Commun. 1, 177–190
12. Friedlander, D. R., Milev, P., Karthikeyan, L., Margolis, R. K., Margolis, R. U., and Grumet, M. (1994) J. Cell Biol. 125, 669–680
13. Hlavin, M. L., and Lemmon, V. (1991) Genomics 11, 416–423
14. Djabali, M., Mattei, M. G., Nguyen, C., Roux, D., Demengeot, J., Denizot, F., Moos, M., Schachner, M., Goridis, C., andordan, B. R. (1990) Genomics 7, 587–593
15. Vits, L., Camp, G. V., Coucke, P., Fransen, E., Boule, K. O., Reynolds, E., Korn, B., Pouskta, A., Wilson, G., Schrander-Stumpel, C., Winter, R. M., Schwartz, C., and Willems, P. J. (1994) Nat. Genet. 7, 408–413
16. Jouvet, M., Rosenthal, A., Armstrong, G., Madalane, J., Steverson, R., Paterson, J., Metzenberg, L., Ionasescu, V., Temple, K., and Kenwrick, S. (1994) Nat. Genet. 7, 402–407
17. Jouvet, M., Mondal, A., Paterson, J., McKewon, C., Fryer, A., Carpenter, N., Hildreng E., Wiaddius, C., and Kenwrick, S. (1995) Am. J. Hum. Genet. 56, 1304–1314
18. Zhao, X., and Siu, C.-H. (1995) J. Biol. Chem. 270, 29413–29421
19. Zhou, H., Fuks, A., Alaraz, A., Blood, T. J., and Stanners, C. P. (1993) J. Cell Biol. 122, 951–960
20. Burgoon, M. P., Grumet, M., Mauro, V., Edelman, G. M., and Cunningham, B. A. (1991) J. Cell Biol. 112, 1017–1029
21. Bieber, A. J., Snow, P. M., Hortsch, M., Patel, N. H., Jacobs, J. R., Traquina, Z. R., Schilling, J., and Goodman, C. S. (1989) J. Cell Biol. 115, 937–945
22. Rao, Y., Wu, X. F., Gariy, J., Rutishauser, U., and Siu, C.-H. (1992) J. Cell Biol. 118, 937–945
23. Rao, Y., Zhao, X., and Siu, C.-H. (1994) J. Biol. Chem. 269, 27540–27548
24. Sandig, M., Rao, Y., and Siu, C.-H. (1994) J. Biol. Chem. 269, 14841–14848
25. Appel, F., Hörm, J., Conscience, J., and Schachner, M. (1993) J. Neurosci. 13, 4764–4775
26. Burgoon, M. P., Haxan, R. B., Phillips, G. R., Crossin, K. L., Edelman, G. M., and Cunningham, B. A. (1995) J. Cell Biol. 130, 733–744
27. Doherty, P., Williams, E., and Walsh, F. S. (1995) Neuron 14, 57–66
28. Isgitz, M. A., Miller, D. R., Soriano, P., and Maness, P. F. (1994) Neuron 12, 873–884
29. Davis, J. O., and Bennett, V. (1994) J. Biol. Chem. 269, 27163–27166
30. Montgomery, A. M. P., Becker, J. C., Siu, C.-H., Lemmon, V. P., Chereces, D. A., Panock, J. D., Zhao, X., and Riefeld, R. A. (1996) J. Cell Biol., in press
31. Hirsch, E., Gullberg, D., Malacz, F., Altruda, F., Silengo, L., and Tarone, G. (1994) Dev. Dynamics 201, 108–120
Differential Effects of Two Hydrocephalus/MASA Syndrome-related Mutations on the Homophilic Binding and Neuritogenic Activities of the Cell Adhesion Molecule L1
Xiaoning Zhao and Chi-Hung Siu

J. Biol. Chem. 1996, 271:6563-6566.
doi: 10.1074/jbc.271.12.6563

Access the most updated version of this article at http://www.jbc.org/content/271/12/6563

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 30 references, 13 of which can be accessed free at http://www.jbc.org/content/271/12/6563.full.html#ref-list-1