

Laminin-database v.2.0: an update on laminins in health and neuromuscular disorders

Daiane C. F. Golbert^{1,2}, Eliane Santana-van-Vliet², Alex S. Mundstein¹, Vicente Calfo¹, Wilson Savino² and Ana Tereza R. de Vasconcelos^{1,*}

¹Bioinformatics Laboratory, National Laboratory of Scientific Computation, Avenue Getúlio Vargas 333, 25651-075, Petrópolis, Rio de Janeiro, Brazil and ²Laboratory on Thymus Research, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Avenue Brasil 4365, 21045-900, Rio de Janeiro, Brazil

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ABSTRACT

The laminin (LM)-database, hosted at <http://www.lm.lncc.br>, was published in the NAR database 2011 edition. It was the first database that provided comprehensive information concerning a non-collagenous family of extracellular matrix proteins, the LMs. In its first version, this database contained a large amount of information concerning LMs related to health and disease, with particular emphasis on the haemopoietic system. Users can easily access several tabs for LMs and LM-related molecules, as well as LM nomenclatures and direct links to PubMed.

The LM-database version 2.0 integrates data from several publications to achieve a more comprehensive knowledge of LMs in health and disease. The novel features include the addition of two new tabs, 'Neuromuscular Disorders' and 'miRNA--LM Relationship'. More specifically, in this updated version, an expanding set of data has been displayed concerning the role of LMs in neuromuscular and neurodegenerative diseases, as well as the putative involvement of microRNAs. Given the importance of LMs in several biological processes, such as cell adhesion, proliferation, differentiation, migration and cell death, this upgraded version expands for users a panoply of information, regarding complex molecular circuitries that involve LMs in health and disease, including neuromuscular and neurodegenerative disorders.

INTRODUCTION

Extracellular matrix (ECM) protein interactions are essential for normal biological processes and when disrupted, they may lead to pathological processes. The laminin

(LM) proteins are heterotrimers of alpha, beta and gamma chains. Five α -, four β - and three γ -chains give rise to at least 16 different protein isoforms in mammals (1–4). LM isoforms can drive numerous effects in cells, including cell adhesion, differentiation, proliferation, survival/apoptosis and migration (5,6). All these biological processes require the participation of integrins, the major receptors for ECM proteins. So far, 11 integrins have been described as LM receptors: $\alpha 1\beta 1$, $\alpha 2\beta 1$, $\alpha 2\beta 2$, $\alpha 3\beta 1$, $\alpha 6\beta 1$, $\alpha 6\beta 4$, $\alpha 7\beta 1$, $\alpha 9\beta 1$, $\alpha v\beta 3$, $\alpha v\beta 5$ and $\alpha v\beta 8$ (7–9).

Several studies have demonstrated the importance of LMs in different types of diseases including cancer, myopathies and neurodegenerative disorders.

Neuromuscular diseases include some of the most devastating disorders that afflict mankind. These disorders may be inherited as autosomal dominant, autosomal recessive or X-linked traits. Although individually rare, collectively neuromuscular diseases have an incidence of 1 in 3000. These disorders refer to diseases that affect any part of the peripheral nervous system and muscle. Therefore, the ultimate effect of neuromuscular disorders is on the ability to perform voluntary movements, leading to significant disability of the motor system, resulting in almost complete paralysis. In the past decade, a large number of human genes causing neuromuscular diseases have been identified. Nevertheless, the underlying pathogenic mechanisms remain largely unclear, limiting the development of targeted therapeutics (10–13).

MicroRNAs (miRNAs) constitute an abundant class of non-coding RNAs involved in regulating post-transcriptional gene expression, predominantly by translational repression. These small RNAs have been identified in animals, plants and viruses. In humans, >1000 miRNA sequences have been found so far (www.mirbase.org) (14,15).

During the past decade, a substantial amount of knowledge has been accumulated regarding the biogenesis of miRNAs. A single miRNA may have broad effects on gene expression networks, such as regulating cell

*To whom correspondence should be addressed. Tel: +55 24 2233 6054; Fax: +55 24 2233 6230; Email: atrv@lncc.br

lineage specificity, cellular functions or stress response. The binding of miRNAs to the 3'-untranslated region to their target mRNAs leads to a decrease in the expression of the target proteins and mRNA destabilization or degradation (14,15). In fact, levels of miRNA inversely correlate with mRNA levels and directly alter mRNA levels (16–18). Emerging evidence points to specific miRNAs targeting and regulating expression of particular proteins that play key roles in pathogenesis. Disease-related dysregulation in miRNA expression indicates roles for miRNAs in pathophysiological states including cardiac hypertrophy, muscle dystrophy, hepatitis infection, diabetes, Parkinson syndrome, haematological malignancies and other types of cancer. In fact, miRNA expression patterns are presently used to classify various tumours (19–25).

miRNAs are also highly expressed in the brain and have important roles in multiple biological processes such as neuronal differentiation, brain development, synapse formation and plasticity and neurodegeneration (26–28). Abnormally increased or decreased expression of various miRNAs may contribute to the pathophysiology of neurodegenerative diseases and psychiatric disorders. Hence, replacement or inhibition of downregulated or upregulated miRNAs, respectively, may be clinically beneficial in the treatment of these disorders (20).

Recently, data showed that LM isoforms are regulated by miRNAs, whose molecular signatures related to LM and neuromuscular disorders were significantly modulated. Furthermore, the regulation by these small RNAs might be crucial for LM participation in several diseases such as Duchenne muscular dystrophy as well as Alzheimer's and Parkinson's diseases (29–37). Thus, it is worthwhile to identify and investigate the functional significance of miRNA expression, in particular, its role related to modulation of LM isoforms or their corresponding receptors. Likewise, the identification of regulatory pathways may provide new avenues of investigation for the development of novel therapies.

The interrelationship between LM and miRNAs appears to play a crucial role in health and disease. In the first version of this database, we presented a number of publications demonstrating the importance of LMs in general, and in particular, the role of these ECM molecules in the haemopoietic system. The first version of the LM-database was published in 2011 (38).

For the LM-database version 2.0, we have extended the original biological information by adding new data concerning LM related to neuromuscular disorders, and miRNA expression and regulation.

CHANGES IN LM-DATABASE VERSION 2.0

Updated database contents

Structurally, the first version of LM-databases' main menu is subdivided into different tabs: LMs, receptor-binding proteins and other related proteins. The option to open each tab into a given LM or LM-related molecule has been maintained. There, the user finds a series of tabs for 'protein', 'gene structure', 'gene expression' and

'tissue distribution' separated by species, comprising *Homo sapiens*, *Mus musculus* and *Rattus norvegicus*.

A powerful design feature of the LM-database version 2.0 is the ability to search datasets to explore the biological aspects of LM in neuromuscular disorders and their relationship with miRNAs. The information retrieved from the literature and inserted into this version was manually annotated and curated. In addition, each record has been linked to a PubMed ID (PMID), which has been directly linked to the corresponding abstract retrieved from PubMed. The whole datasets are distributed into two new tabs: the 'Neuromuscular Disorders' tab, arranged in the main menu, and the 'miRNA–LM Relationship' tab inserted for each molecule placed in the list of proteins.

The LM-database Web site is interlinked with other publicly available databases, e.g. 2D gel, retrieved from UniProt (1), Protein Data Bank (PDB) (2), protein domains in Pfam (3) and Interpro (4), as well as Ensembl (5). Additionally, miRNA database links were added in both 'Neuromuscular Disorders' and 'miRNA–LM Relationship' tabs. This enables users to follow links and retrieve relevant information from other sources related to the biological area of interest.

Neuromuscular disorders tab

This tab comprises a collection of information regarding the involvement of LMs in neurodegenerative disorders. Users can find a set of topics such as 'Myopathies', 'Infectious Neuropathies' and 'Neurodegenerative Diseases'. When clicking onto a chosen topic, users gain access to annotated information concerning specific groups of neuromuscular disorders. Additionally, to facilitate access to data, users can find a number of database links related to brain disorders inside this tab.

LM–miRNA Relationship tab

Altered expression of certain miRNAs in the brain of patients with neurodegenerative diseases has been described, and a growing amount of publications have shown that ECM protein expressions are involved in several neuromuscular diseases. (34,35,39–41). This has prompted us to create the 'LM–miRNA Relationship' tab. For each LM or LM-related molecule presented in the list of proteins, appropriate information concerning miRNA expression and regulation has been provided.

It is important to note that neurodegenerative disease-specific miRNA contents were also incorporated in the 'Neuromuscular Disorders' tab. In addition, this tab contains a list of links to a compilation of miRNA databases, Web sites and servers, which allows users to access the contents of several miRNA databank sites.

CONCLUDING REMARKS

The LM-database was the first of its kind that collected a substantial amount of data concerning a non-collagenous extracellular protein family. Currently, it is the most comprehensive source of information related to LMs, and it provides an overview on LMs as well as LM receptor expression in physiology and pathophysiology.

We have updated information on LMs and integrins related to neuromuscular disorders to LM-database version 2.0. Considering the prominent role of miRNAs in regulating gene expression, it was considered essential to include detailed information concerning miRNA expression related to LMs and neuromuscular diseases. This was made possible by the addition of two navigation tabs, which give direct access to current data from the literature. The amount of available information in LM-database version 2.0 has been largely improved and expanded compared with the first version. Finally, the growing body of publications concerning miRNAs related to LMs and neuromuscular diseases further highlights the relevance of this database. Overall, we plan to update LM-database continuously with new data from the literature as well as to look for more improvements.

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REFERENCES

1. Miner, J.H. and Yurchenco, P.D. (2004) Laminin functions in tissue morphogenesis. *Annu. Rev. Cell Dev. Biol.*, **20**, 255–284.
2. Miner, J.H. (2008) Laminins and their roles in mammals. *Microsc. Res. Tech.*, **71**, 349–356.
3. Aumailley, M., Bruckner-Tuderman, L., Carter, W.G., Deutzmann, R., Edgar, D., Ekblom, P., Engel, J., Engvall, E., Hohenester, E., Jones, J.C. *et al.* (2005) A simplified laminin nomenclature. *Matrix Biol.*, **24**, 326–332.
4. Robert, E., Burgeson, R.E., Chiquet, M., Deutzmann, R., Ekblom, P., Engel, J., Kleinman, H., Martin, G.R., Meneguzzi, G., Paulsson, M. *et al.* (1994) A new nomenclature for the laminins. *Matrix Biol.*, **14**, 209–211.
5. Savino, W., Dalmau, S.R. and Dealmeida, V.C. (2000) Role of extracellular matrix-mediated interactions in thymocyte migration. *Dev. Immunol.*, **7**, 279–291.
6. Silva-Barbosa, S.D., Butler-Browne, G.S., de Mello, W., Riederer, I., Di Santo, J.P., Savino, W. and Mouly, V. (2008) Human myoblast engraftment is improved in laminin-enriched microenvironment. *Transplantation.*, **85**, 566–575.
7. Durbeej, M. (2010) Laminins. *Cell Tissue Res.*, **339**, 259–268.
8. Belkin, A.M. and Stepp, M.A. (2000) Integrins as receptors for laminins. *Microsc. Res. Tech.*, **51**, 280–301.
9. Barczyk, M., Carracedo, S. and Gullberg, D. (2010) Integrins. *Cell Tissue Res.*, **339**, 269–280.
10. Laing, N.G. (2012) Genetics of neuromuscular disorders. *Crit. Rev. Clin. Lab. Sci.*, **49**, 33–48.
11. Farrer, M.G. (2006) Genetics of Parkinson disease: paradigm shifts and future prospects. *Nat. Rev. Genet.*, **7**, 306–318.
12. Bertram, L. and Tanzi, R.E. (2008) Thirty years of Alzheimer's disease genetics: the implications of systematic meta-analyses. *Nat. Rev. Neurosci.*, **9**, 768–778.
13. Davies, K.E. and Nowak, K.J. (2006) Molecular mechanisms of muscular dystrophies: old and new players. *Nat. Rev. Mol. Cell Biol.*, **7**, 762–773.
14. Bartel, D.P. (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*, **116**, 281–297.
15. Bartel, D.P. (2009) MicroRNAs: target recognition and regulatory functions. *Cell*, **136**, 215–233.
16. Sood, P., Krek, A., Zavolan, M., Macino, G. and Rajewsky, N. (2006) Cell-type-specific signatures of microRNAs on target mRNA expression. *Proc. Natl. Acad. Sci. USA*, **103**, 2746–2751.
17. Lim, L.P., Lau, N.C., Garrett-Engele, P., Grimson, A., Schelter, J.M., Castle, J., Bartel, D.P., Linsley, P.S. and Johnson, J.M. (2005) Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature*, **433**, 769–773.
18. Guo, H., Ingolia, N.T., Weissman, J.S. and Bartel, D.P. (2010) Mammalian microRNAs predominantly act to decrease target mRNA levels. *Nature*, **466**, 835–840.
19. Lukiw, W.J., Andreeva, T.V., Grigorenko, A.P. and Rogae, E.I. (2012) Studying microRNA function and dysfunction in Alzheimer's disease. *Front. Genet.*, **3**, 327.
20. Akerblom, M., Sachdeva, R. and Jakobsson, J. (2012) Functional studies of microRNAs in neural stem cells: problems and perspectives. *Front. Neurosci.*, **6**, 14.
21. Im, H.I. and Kenny, P.J. (2012) MicroRNAs in neuronal function and dysfunction. *Trends Neurosci.*, **35**, 325–334.
22. Wang, J. and Yang, X. (2012) The function of miRNA in cardiac hypertrophy. *Cell Mol. Life Sci.*, **69**, 3561–3570.
23. Eisenberg, I., Alexander, M.S. and Kunkel, L.M. (2009) miRNAs in normal and diseased skeletal muscle. *J. Cell Mol. Med.*, **13**, 2–11.
24. Guay, C. and Regazzi, R. (2013) Circulating microRNAs as novel biomarkers for diabetes mellitus. *Nat. Rev. Endocrinol.*, **9**, 513–521.
25. Gupta, A., Swaminathan, G., Martin-Garcia, J. and Navas-Martin, S. (2012) MicroRNAs, hepatitis C virus, and HCV/HIV-1 co-infection: new insights in pathogenesis and therapy. *Viruses*, **4**, 2485–2513.
26. Mendell, J.T. (2005) MicroRNAs: critical regulators of development, cellular physiology and malignancy. *Cell Cycle*, **4**, 1179–1184.
27. Cheng, L.C., Pastrana, E., Tavazoie, M. and Doetsch, F. (2009) MiR-124 regulates adult neurogenesis in the subventricular zone stem cell niche. *Nat. Neurosci.*, **12**, 399–408.
28. Fiore, R., Siegel, G. and Schratz, G. (2008) MicroRNA function in neuronal development, plasticity and disease. *Biochim. Biophys. Acta.*, **1779**, 471–478.
29. Schaefer, A., O'Carroll, D., Tan, C.L., Hillman, D., Sugimori, M., Llinas, R. and Greengard, P. (2007) Cerebellar neurodegeneration in the absence of microRNAs. *J. Exp. Med.*, **204**, 1553–1558.
30. Bushati, N. and Cohen, S.M. (2008) MicroRNAs in neurodegeneration. *Curr. Opin. Neurobiol.*, **18**, 292–296.
31. Cao, X., Pfaff, S.L. and Gage, F.H. (2007) A functional study of miR-124 in the developing neural tube. *Genes Dev.*, **21**, 531–536.
32. Wewer, U.M., Thornell, L.E., Loechel, F., Zhang, X., Durkin, M.E., Amato, S., Burgeson, R.E., Engvall, E., Albrechtsen, R. and Virtanen, I. (1997) Extrasynaptic location of laminin beta 2 chain in developing and adult human skeletal muscle. *Am. J. Pathol.*, **151**, 621–631.
33. Cacchiarelli, D., Legnini, I., Martone, J., Cazzella, V., D'Amico, A., Bertini, E. and Bozzoni, I. (2011) MiRNAs as serum biomarkers for Duchenne muscular dystrophy. *EMBO Mol. Med.*, **3**, 258–265.
34. Eisenberg, I., Eran, A., Nishino, I., Moggio, M., Lamperti, C., Amato, A.A., Lidov, H.G., Kang, P.B., North, K.N., Mitrani-Rosenbaum, S. *et al.* (2007) Distinctive patterns of microRNA expression in primary muscular disorders. *Proc. Natl. Acad. Sci. USA*, **104**, 17016–17021.

35. Valastyan,S. and Weinberg,R.A. (2011) Roles for microRNAs in the regulation of cell adhesion molecules. *J. Cell Sci.*, **124**(Pt. 7), 999–1006.
36. Enciu,A.M., Popescu,B.O. and Gheorghisan-Galateanu,A. (2012) MicroRNAs in brain development and degeneration. *Mol. Biol. Rep.*, **39**, 2243–2252.
37. Kasai,S., Urushibata,S., Hozumi,K., Yokoyama,F., Ichikawa,N., Kadoya,Y., Nishi,N., Watanabe,N., Yamada,Y. and Nomizu,M. (2007) Identification of multiple amyloidogenic sequences in laminin-1. *Biochemistry*, **46**, 3966–3974.
38. Golbert,D.C., Linhares-Lacerda,L., Almeida,L.G., Correa-de-Santana,E., de Oliveira,A.R., Mundstein,A.S., Savino,W. and de Vasconcelos,A.T. (2011) Laminin database: a tool to retrieve high-throughput and curated data for studies on laminins. *Nucleic Acids Res.*, **39**, D320–D323.
39. Flanagan,L.A., Rebaza,L.M., Derzic,S., Schwartz,P.H. and Monuki,E.S. (2006) Regulation of human neural precursor cells by laminin and integrins. *J. Neurosci. Res.*, **83**, 845–856.
40. Hall,P.E., Lathia,J.D., Caldwell,M.A. and Ffrench-Constant,C. (2008) Laminin enhances the growth of human neural stem cells in defined culture media. *BMC Neurosci.*, **9**, 71.
41. Matsuzawa,M., Weight,F.F., Potember,R.S. and Liesi,P. (1996) Directional neurite outgrowth and axonal differentiation of embryonic hippocampal neurons are promoted by a neurite outgrowth domain of the B2-chain of laminin. *Int. J. Dev. Neurosci.*, **14**, 283–295.