Biophysical analysis of a lethal laminin alpha-1 mutation reveals altered self-interaction

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Abstract

Laminins are key basement membrane molecules that influence several biological activities and are linked to a number of diseases. They are secreted as heterotrimeric proteins consisting of one α, one β, and one γ chain, followed by their assembly into a polymer-like sheet at the basement membrane. Using sedimentation velocity, dynamic light scattering, and surface plasmon resonance experiments, we studied self-association of three laminin (LM) N-terminal fragments α-1 (hLM α-1 N), α-5 (hLM α-5 N) and β-3 (hLM β-3 N) originating from the short arms of the human laminin αβγ heterotrimer. Corresponding studies of the hLM α-1 N C49S mutant, equivalent to the larval lethal C56S mutant in zebrafish, have shown that this mutation causes enhanced self-association behavior, an observation that provides a plausible explanation for the inability of laminin bearing this mutation to fulfill functional roles in vivo, and hence for the deleterious pathological consequences of the mutation on lens function.

Introduction

Laminins (LM) are highly glycosylated basement membrane proteins built from one α, one β and one γ chain that are linked covalently by disulfide bonds between coiled-coil domains [2,8,28] and assemble into 18 isoforms [10,17]. Each subunit comes in a variety of states; there are five isoforms for α (denoted α1 to α5), three different types for β (β1 to β3) and three variants for γ (γ1 to γ3) [36]. A common feature of the three types of laminin chains is the presence of an N-terminal short arm containing two globular domains (domains LN (formerly VI) and L4a/LF/L4 (formerly IV)), followed by a series of laminin-type epidermal growth factor-like domains [3] — as represented schematically in Fig. 1. Exceptions are the laminin α4 and a spliced version of laminin α3 (α3A) that lack the N-terminal short arm [36]. The N-terminal short arms merge into the laminin long arm, a three-stranded left-handed coiled coil. The α laminin chain continues in a tandem array of five laminin globular (LG) domains after the coil [7]. Basement membrane assembly begins with the polymerization of laminin into a cell-associated network [58]. A key step in this process that is mediated by the N-terminal domains of the three short chains of the laminin αβγ-heterotrimer has been described as the “three arms interaction model” [17]. The current work focuses on two truncated forms of the N-terminal

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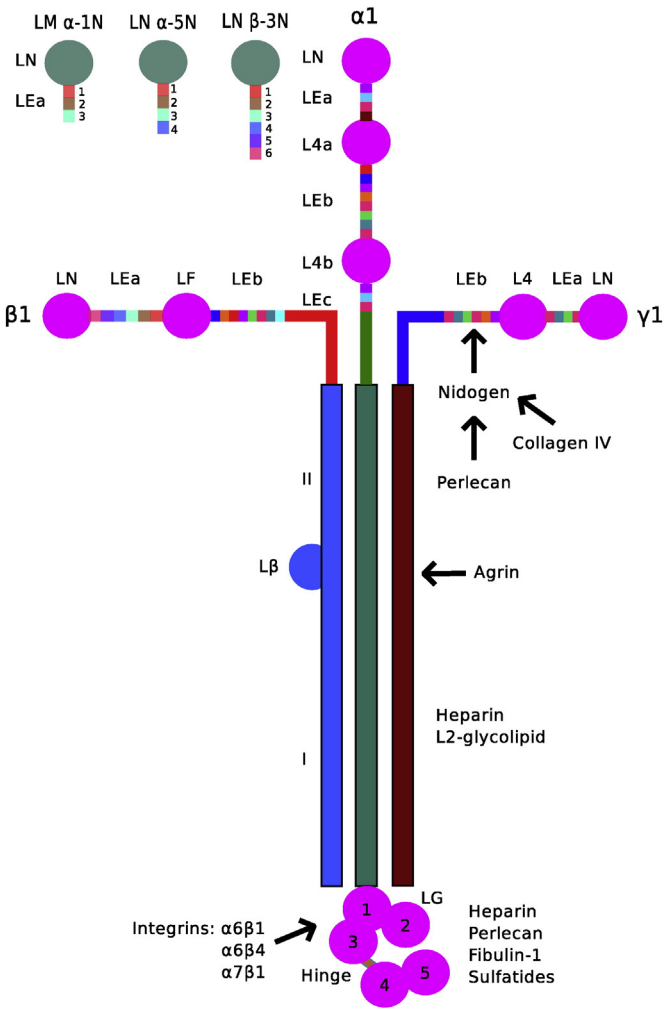

Fig. 1. Schematic representation of the human αβγ heterotrimer showing the location of the three short-arm segments (LM α-1 N, LM α-5 N and LM β-3 N) being investigated.

region of the LM α-short arm, designated LM α-1 N and LM α-5 N, that comprise the globular LN domain and three (α-1) or four (α-5) LEa domains, and also on a corresponding segment of the LM β-short arm, (designated LM β-3 N), that includes the globular LN domain and six LEa domains. Their location within the laminin αβγ-heterotrimer is indicated in Fig. 1.

Interest in LM α-1 stems from its involvement in a number of physiological and pathological processes [10]. Ning et al. [38] demonstrated that the absence of LM α-1 results in increased proliferation of mesangial cells in the kidney and increased TGF-β1 mediated Smad2 phosphorylation. LM α-1 is required for the development and organization of the cerebellum and for the migration of granular cells [16,21]. An ablation of LM α-1, which is an essential component of laminin-111 heterotrimer that forms a highly specialized and thick extra-embryonic Reichert's membrane is embryonic lethal [35]. The subunit also regulates neuronal polarity and directional guidance [56], and is required for lens development in zebrafish [60]. A mutation in C56 to serine of LM α-1 N in zebrafish leads to defects with the development of lens, cornea, and retina resulting in lens degeneration and focal cornea dysplasia — a mutation also causes death of larvae by 12 days [50]. This cysteine and other cysteine residues are conserved across species as signified by sequence alignment (Supplementary Fig. 1).

LM α-5 influences several biological processes including tissue patterning, organogenesis and embryogenesis, and its absence has been linked to limb defects in mouse [52]. Recently, its role in mouse placental labyrinth development and formation has been demonstrated [25]. It is also crucial for the establishment and maintenance of the glomerular filtration barrier in murine kidneys [15,34], as well as for murine lung development [37]. Overexpression of LM β-3 in colorectal cancer has been linked with chemoresistance of cancer patients [12].

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