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Review

Engineered mutations in fibrillin-1 leading to Marfan syndrome act at the protein, cellular and organismal levels

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ABSTRACT

Fibrillins are the major components of microfibrils in the extracellular matrix of elastic and non-elastic tissues. They are multi-domain proteins, containing primarily calcium binding epidermal growth factor-like (cbEGF) domains and 8-cysteine/transforming growth factor-beta binding protein-like (TB) domains. Mutations in the fibrillin-1 gene give rise to Marfan syndrome, a connective tissue disorder with clinical complications in the cardiovascular, skeletal, ocular and other organ systems. Here, we review the consequences of engineered Marfan syndrome mutations in fibrillin-1 at the protein, cellular and organismal levels.

Representative point mutations associated with Marfan syndrome in affected individuals have been introduced and analyzed in recombinant fibrillin-1 fragments. Those mutations affect fibrillin-1 on a structural and functional level. Mutations which impair folding of cbEGF domains can affect protein trafficking. Protein folding disrupted by some mutations can lead to defective secretion in mutant fibrillin-1 fragments, whereas fragments with other Marfan mutations are secreted normally. Many Marfan mutations render fibrillin-1 more susceptible to proteolysis. There is also evidence that some mutations affect heparin binding. Few mutations have been further analyzed in mouse models. An extensively studied mouse model of Marfan syndrome expresses mouse fibrillin-1 with a missense mutation (p.C1039G). The mice display similar characteristics to human patients with Marfan syndrome. Overall, the analyses of engineered mutations leading to Marfan syndrome provide important insights into the pathogenic molecular mechanisms exerted by mutated fibrillin-1.

Contents

Abbreviations: BMP2, bone morphogenetic protein 2; cbEGF domain, calcium binding epidermal growth factor-like domain; EGF domain, epidermal growth factor-like domain; FBN1, fibrillin-1 gene; HEK 293 cells, human embryonic kidney 293 cells; Hyb domain, Hybrid domain; kDa, kilodalton; MMP, matrix metalloproteinase; PTC, premature termination codon; TB domain, transforming growth factor-beta-binding protein-like domain; TGF-β, transforming growth factor-beta.

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1. Introduction

1.1. Marfan syndrome

Marfan syndrome is a heritable autosomal-dominant connective tissue disorder with a prevalence of 2–3 in 10,000 individuals [1]. It affects many different organ systems, most importantly large blood vessels, bones, and eyes. Major clinical manifestations include aortic aneurysms and dissections, long bone overgrowth, scoliosis, and ectopia lentis. Aortic aneurysms and dissections can be life-threatening if not monitored and treated adequately. The genetic defect that leads to Marfan syndrome was mapped to chromosome 15 where the fibrillin-1 gene (*FBN1*) is located [2]. Mutations in this gene have been shown to give rise to Marfan syndrome [3] (see Section 1.3). More than 1800 different mutations have been identified in *FBN1*, most of which are associated with the clinical phenotype of Marfan syndrome (http://www.umd.be/FBN1) [4]. Marfan syndrome is characterized by a wide inter- and intrafamilial phenotypic variability. Therefore, it is crucial to better understand the structural and functional consequences of mutations on the fibrillin-1 protein.

1.2. Structure and function of fibrillin-1

1.2.1. Fibrillin-1 is a multidomain protein

Fibrillin-1 is a member of the fibrillin family of ~350 kDa glycoproteins that are the major components of microfibrils in the extracellular matrix of elastic and non-elastic tissues [5]. The fibrillin family encompasses three highly homologous proteins in higher vertebrates, fibrillin-1, fibrillin-2 and fibrillin-3. Fibrillin-1 expression persists into adulthood, whereas fibrillin-2 and -3 are mainly expressed during development [6–8].

Fibrillin-1 is a multi-domain protein mainly composed of epidermal growth factor-like (EGF) domains (Fig. 1) [9]. 43 out of the 47 EGF-like domains in fibrillin-1 contain the calcium binding (cb) consensus sequence D/N-X-D/N-E/Q-X_m-D/N-X_n-Y/F with *m* and *n* being variable numbers of amino acid residues (Fig. 2). The residues of this consensus sequence either directly ligate calcium or stabilize the calcium binding site [10–12]. Calcium binding to fibrillin-1 is important for the structure and function of the protein. It provides structural stabilization [13–15], protects the protein against proteolysis [16], and controls interaction with a variety of extracellular matrix components [17–21]. Calcium binding together with hydrophobic packing interactions is a key structural component that restricts interdomain flexibility and allows for the characteristic rigid rod-like shape of fibrillin-1 [13,22,23]. Each cbEGF domain is stabilized by the formation of three disulfide bonds between the six cysteine residues in each domain in a C1–C3, C2–C4 and C5–C6 pattern [13,24].

The cbEGF domains are interspersed mainly by two other types of domains, transforming growth factor beta binding protein-like (TB) and hybrid (Hyb) domains [9]. TB domains occur seven times in vertebrate fibrillins, and contain eight cysteine residues that form disulfide bonds in a C1–C3, C2–C6, C4–C7 and C5–C8 pattern [25]. Hyb domains are found only twice in all mammalian fibrillins. They show similarities with TB domains in their N-terminal region and with cbEGF domains in their C-terminal region [9,26,27]. Other protein domains in fibrillin-1 include the unique N-and C-terminal domains, as well as a proline-rich domain close to the N-terminus.

1.2.2. Fibrillin-1 constitutes the core of microfibrils

Fibrillins are the major backbone components of microfibrils in elastic and non-elastic tissues [5]. Microfibrils confer structural integrity, for example in ciliary zonules of the ocular system or along basement zones in various tissues such as skin and kidney [28,29]. In elastic tissues including blood vessels, lung and skin, microfibrils act as a scaffold for the deposition of tropoelastin, a key regulatory mechanism in elastogenesis [30]. Importantly, microfibrils play a major role in regulating the bioavailability of growth factors of the transforming growth factor beta (TGF-β)/bone morphogenetic protein (BMP) family. Dysregulation of TGF-β is an important contributor to the pathogenesis in Marfan syndrome and related disorders [31]. This molecular phenotype can be antagonized and rescued in a Marfan mouse model by losartan, an angiotensin II type 1 receptor blocker [32]. The beneficial effects of losartan in Marfan patients were analyzed in various clinical trials [33,34]. However, the largest study so far with over 600 children and young adults affected with Marfan syndrome showed no significant difference between the treatment with losartan and atenolol in regard to the rate of aortic dilatation over a period of 3 years [35]. Atenolol is a beta-blocker that has been used for many years as a standard care for patients with Marfan syndrome [36].

1.3. Mutations in fibrillin-1

Mutations in fibrillin-1 give rise to a number of connective tissue disorders, collectively termed type I fibrillinopathies. The most common disorders caused by mutations in fibrillin-1 are various forms of Marfan syndrome, including the common classical form, the severe so-called "neonatal" form with early disease onset, and the progeroid form [3,37,38]. Other disorders caused by fibrillin-1 mutations include dominant Weill–Marchesani syndrome [39], acromicric and geleophysic dysplasia [40], dominant ectopia lentis [41], and stiff skin syndrome [42]. Although all of these disorders arise from mutations in fibrillin-1, they manifest with wide phenotypic differences. For example, patients with Marfan syndrome are typically characterized by long bone overgrowth, ectopia lentis and joint hyperflexibility, whereas dominant Weill–Marchesani syndrome presents with short stature, spherophakia with glaucoma, and joint stiffness.

A large genotype-phenotype study correlated the position of *FBN1* mutations with the associated clinical features and severity