GENETICS OF STRUCTURAL SKIN DISORDERS

The Complexity of Elastic Fiber Biogenesis: The Paradigm of Cutis Laxa

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INTRODUCTION

Cutis laxa (CL) is a skin disorder characterized by prematurely redundant, pendulous, inelastic, and wrinkled skin (Berk *et al.*, 2012), and is associated with variable involvement of other organs. Both acquired and inherited forms of CL exist and the latter show extensive locus heterogeneity and congenital onset. The main histopathologic and ultrastructural anomalies in the skin of patients with CL are paucity, fragmentation, or disorganization of the dermal elastic fibers (Figure 1a). Studies of inherited forms of CL have uncovered a growing network of genes (Table 1). On the basis of congenital nature of most inherited forms of CL, these genes are necessary for elastic fiber biogenesis and are distinct from genes involved in the homeostatic maintenance such as those inhibiting ectopic calcification of the elastic fibers (Li and Uitto, 2012).

ELASTIC FIBER NETWORK

The elastic fibers consist of microfibrils, elastin, and associated proteins, and show tissue-specific organization (Kielty, 2006). In the skin, they are organized in horizontally undulating deep dermal elastic fibers (Figure 1b, arrowhead), connected to thinner, vertical elaunin fibers, which show a candelabra-like branching pattern toward the dermal–epidermal junction (Figure 1b, arrow). The elaunin fibers further branch and terminate in oxytalan fibers, which are composed mostly of microfibrils and anchor the network to the dermal side of the basement membrane. The skin phenotype of patients with CL suggests that the elastic fiber system is not required for the anchoring of the epidermis to the dermis (which is disrupted in blistering diseases) (Bruckner-Tuderman and Has, 2012), but rather appears to couple the growth of the skin to the rest of the body.

MOLECULAR BASIS OF CL

Studies on X-linked CL provided the first insight in to the molecular basis of this disease by showing reduced activity of lysyl oxidase, an enzyme required of the cross-linking of both

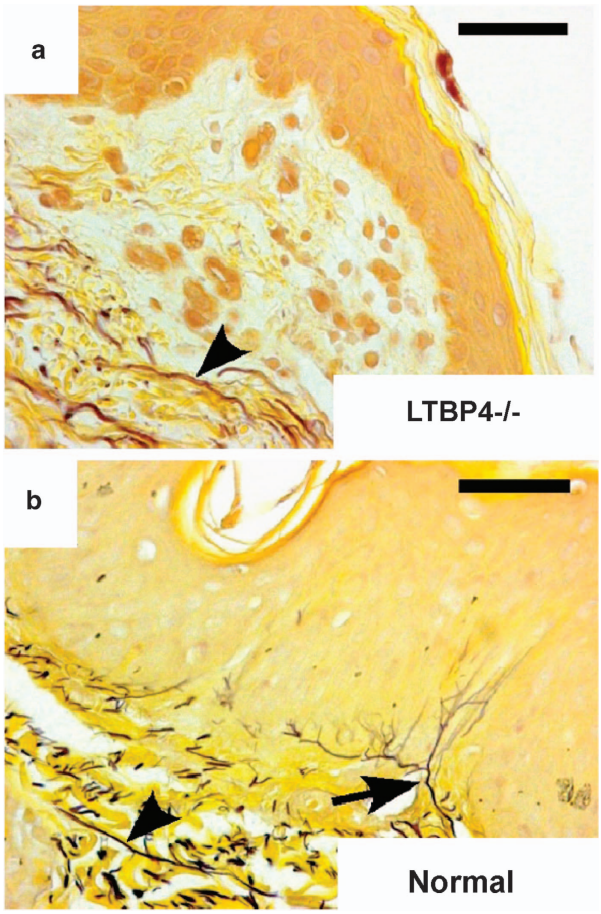

Figure 1. Elastic fiber abnormalities in cutis laxa. Hart's elastin stain of a skin biopsy specimen from (**a**) a patient with ARCL1C/URDS caused by a homozygous mutation in *LTBP4*, and from (**b**) a normal control individual. Deep dermal elastic fibers (arrowhead) are diminished and show altered staining in the patient. The elastic fibers in the superficial dermis are missing in the patient but are present in the normal control (arrow). Magnification bars: 20 µm. Reproduced from (Urban *et al.*, 2009).

Table 1. Genes for cutis laxa and related conditions

Disease	Distinguishing clinical features	Mutated genes
ADCL	Pulmonary and cardiovascular manifestations absent, milder or later onset	*ELN*
ARCL1A	Arterial tortuosity, lethal pulmonary hypertension, bone fragility	*FBLN4/EFEMP2*
ARCL1B	Supravalvular aortic stenosis, lethal developmental emphysema	*FBLN5*
ARCL1C/URDS	Severe gastrointestinal and urinary malformations, lethal developmental emphysema mild cardiovascular involvement	*LTBP4*
ARCL2A	Growth and developmental delay, abnormal glycosylation of serum proteins	*ATP6V0A2*
ARCL2B	Growth and developmental delay, triangular face, normal glycosylation	*PYCR1*
XLCL	Occipital exostoses, pili torti	*ATP7A*
DBS/ARCL3	Corneal clouding, athetoid movements	*ATP6V0A2, PYCR1, ALDH18A1*
GO	Bone fragility, short stature	*GORAB*
MACS	Macrocephaly, alopecia, scoliosis	*RIN2*

Abbreviations: ADCL, autosomal dominant cutis laxa; ARCL, autosomal recessive cutis laxa; ATS, arterial tortuosity syndrome; DBS, DeBarsy syndrome; GO, geroderma osteodysplastica; MACS, macrocephaly-alopecia-cutis laxa-scoliosis syndrome; URDS, Urban–Rifkin–Davis syndrome; XLCL, X-linked cutis laxa.

elastin and collagens (Byers *et al.*, 1980) (Byers and Murray, 2012). X-linked CL was recognized to be identical to occipital horn syndrome, found to be allelic to Menkes disease, and to be caused by mutations in the copper transporter *ATP7A* (Kaler *et al.*, 1994). The activity of lysyl oxidase and several other enzymes (tyrosinase, dopamine β-hydroxylase) is dependent on copper.

Genes encoding known components of the elastic fibers became candidate genes for CL. First, mutations in the *elastin* gene (*ELN*) were identified in autosomal dominant CL (ADCL) (Tassabehji *et al.*, 1998; Zhang *et al.*, 1999), characterized by a relatively mild course, but sometimes associated with aortic aneurysm and pulmonary emphysema (Urban *et al.*, 2005; Szabo *et al.*, 2006). The *ELN* mutations responsible for ADCL result in a protein deficient in interacting with microfibrils but with increased self-association (Callewaert *et al.*, 2011). Incorporation of mutant elastin into the elastic fibers results in reduced compliance of tissues (Hu *et al.*, 2010), consistent with a dominant-negative mechanism.

Autosomal recessive CL type 1 (ARCL1) is a disease with high infantile and childhood mortality because of severe developmental emphysema or vascular defects. The identification of all three genes responsible for ARCL1 was greatly aided by the similarity in phenotype to knockout mouse models. ARCL1A associated with arterial tortuosity, aneurysms, and bone fragility is caused by mutations in *fibulin-4* (*FBLN4/EFEMP2*) (Hucthagowder *et al.*, 2006). ARCL1B is distinguished by the presence of supravalvular aortic stenosis, and *fibulin-5* (*FBLN5*) is mutated in affected individuals (Loeys *et al.*, 2002). Finally, ARCL1C, a disease with severe gastrointestinal and urinary involvement, is a result of mutations in the gene for the *latent transforming growth factor-beta-binding protein 4* (*LTBP4*) (Urban *et al.*, 2009).

All the three genes for ARCL1 (*FBLN4, FBLN5, LTBP4*) encode secreted, multi-adhesive proteins required for the hierarchical, cell-directed process of elastic fiber assembly (Czirok *et al.*, 2006). Transforming growth factor-beta (TGFβ) signaling is a common downstream pathway activated in ADCL (Hu *et al.*, 2010; Callewaert *et al.*, 2011), ARCL1A (Renard *et al.*, 2010), and ARCL1C (Urban *et al.*, 2009), and may provide a useful therapeutic target in the future.

ARCL type 2 (ARCL2) and related syndromes are associated with growth and developmental delay. Positional cloning of several causative genes has uncovered intracellular molecular pathways previously not connected to elastic fiber biogenesis. ARCL2A, characterized by abnormal glycosylation of serum proteins, is a result of mutations in the *ATP6V0A2* gene, encoding the A2 subunit of the vesicular proton pump (Kornak *et al.*, 2008).

Patients with ARCL2B have triangular face and prematurely aged appearance and carry mutations in the *pyrroline-5-carboxylate reductase* (*PYCR1*) gene, encoding a mitochondrial enzyme involved in the proline biosynthetic pathway (Guernsey *et al.*, 2009). Mutations in this gene result in altered mitochondrial morphology and increased oxidative stress (Reversade *et al.*, 2009).

De Barsy syndrome is an autosomal recessive disease overlapping with the ARCL2 phenotype, but distinguished by the presence of corneal opacities and cataracts. Mutations in *ATP6V0A2* (Kornak *et al.*, 2008), *PYCR1* (Reversade *et al.*, 2009), or the gene for *Δ^1-pyrroline-5-carboxylate synthase* (*ALDH18A1*) (Skidmore *et al.*, 2011), encoding another mitochondrial proline biosynthetic enzyme, have been described in patients with De Barsy syndrome.

Gerodermia osteodysplastica is a related disease characterized by marked bone fragility and caused by recessive mutations in the *GORAB* gene (Hennies *et al.*, 2008), which encodes a binding partner of the Rab6 guanosine triphosphatase involved in vesicular trafficking. Finally, CL with macrocephaly, alopecia, and scoliosis is a recessive disease linked to mutations in the *RIN2* gene (Basel-Vanagaite *et al.*, 2009), encoding a interactor of the Rab5 guanosine triphosphatase, another small G protein required for vesicle sorting and trafficking.

These gene identification studies have highlighted the essential role of the secretory pathway in elastic fiber biogenesis. For example, the secretion of the elastin precursor, tropoelastin,

is impaired in cells with *ATP6V0A2* mutations leading to accumulation of tropoelastin in Golgi vesicles (Hucthagowder *et al.*, 2009). Conversely, in cells with RIN2 mutations the production of microfibrils and fibulin-5 appears to be disrupted (Basel-Vanagaite *et al.*, 2009). These discoveries support the emerging notion that appropriate intracellular sorting and routing of individual elastic fiber proteins, or groups of proteins are essential for normal extracellular fiber assembly. Whether preassembly (or micro-assembly) of these components occurs in particular secretory compartments will be an interesting question to address.

Another surprising set of molecules implicated in elastogenesis are mitochondrial enzymes of the proline biosynthesis pathway. Both elastin and collagens are proline-rich proteins, and thus their synthesis may be particularly sensitive to the availability of this amino acid. However, not all disease-causing *ALDH18A1* mutations affect the flux through the proline biosynthetic pathway (Bicknell *et al.*, 2008), suggesting that this enzyme, and by extension possibly *PYCR1* as well, may have another function more relevant to the disease mechanism. Discovery of how mitochondrial and secretory proteins orchestrate an intracellular milieu conducive to elastic fiber assembly in the extracellular space will open up exciting novel areas of research and new avenues for interventions to treat disorders of the elastic fiber system.

CONCLUSIONS

The phenotypes and associated molecular defects in CL highlight the integration of molecular networks required for elastic fiber biogenesis with pathways associated with other structural skin disorders. Several forms of CL show joint laxity, a hallmark of Ehlers–Danlos syndrome (Byers and Murray, 2012), and *RIN2* mutations result in a phenotype that appears to be intermediate between CL and Ehlers–Danlos syndrome (Syx *et al.*, 2010). Sparse hair has been described in several CL syndromes including patients with *ELN* mutations (Szabo *et al.*, 2006). Moreover, *RIN2* mutations result in alopecia (Basel-Vanagaite *et al.*, 2009). Thus, the elastic fiber system may be connected to the molecular network disrupted in structural hair disorders (Harel and Christiano, 2012).

CONFLICT OF INTEREST

The author states no conflict of interest.

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