Recombinant laminin fragments endowed with collagen-binding activity: A tool for conferring laminin-like cell-adhesive activity to collagen matrices

Ryoko Sato-Nishiuchi [a], Shaoliang Li [a], Fumi Ebisu [a,b] and Kiyotoshi Sekiguchi [a,b]

***a* - Division of Matrixome Research and Application,** *Institute for Protein Research, Osaka University, Suita, Osaka, Japan*
***b* - Division of Research and Development,** *Matrixome Inc., Suita, Osaka, Japan*

Correspondence to Kiyotoshi Sekiguchi: *at: Division of Matrixome Research and Application, Institute for Protein Research, Osaka University, 3-2 Yamadaoka, Suita, Osaka 565-0871, Japan. sekiguch@protein.osaka-u.ac.jp*
http://dx.doi.org/10.1016/j.matbio.2017.08.001

Abstract

Laminins are major components of basement membranes that sustain a wide variety of stem cells. Among 15 laminin isoforms, laminin-511 and its E8 fragment (LM511E8) have been shown to strongly promote the adhesion and proliferation of human pluripotent stem cells. The aim of this study was to endow the cell-adhesive activity of laminin-511 on collagen matrices, thereby fabricating collagen-based culture scaffolds for stem cells with defined composition. To achieve this goal, we utilized the collagen-binding domain (CBD) of fibronectin to immobilize LM511E8 on collagen matrices. CBD was attached to the N-termini of individual laminin chains (α5E8, β1E8, γ1E8), producing LM511E8s having one, two, or three CBDs. While LM511E8 did not bind to collagen, CBD-attached LM511E8s (CBD-LM511E8s) exhibited significant collagen-binding activity, dependent on the number of attached CBDs. Human iPS cells were cultured on collagen-coated plates preloaded with CBD-LM511E8s. Although iPS cells did not attach or grow on collagen, they robustly proliferated on CBD-LM511E8-loaded collagen matrices, similar to the case with LM511E8-coated plates. Importantly, iPS cells proliferated and yielded round-shaped colonies even on collagen gels preloaded with CBD-LM511E8s. These results demonstrate that CBD-attached laminin E8 fragments are promising tools for fabrication of collagen-based matrices having the cell-adhesive activity of laminins.

Introduction

Collagens are the most abundant extracellular matrix (ECM) proteins in vertebrates. In most connective tissues, collagen fibrils and their networks constitute the majority of the ECM and form a highly organized 3D scaffold surrounding cells [1]. Collagens have been widely used in tissue engineering as scaffolds for tissue regeneration and drug delivery, because of their excellent gel-forming abilities and biodegradability [2]. Various types of collagen-based scaffolds, e.g., sponges, hydrogels, and membranes, have been developed as scaffolds for medical applications [1]. Furthermore, conjugation of a variety of bioactive molecules to collagen-based scaffolds has been attempted using elastin [3], glycosaminoglycans [4], or Arg-Gly-Asp (RGD) motif-containing cell-adhesive peptides, well-known ligands for the integrin family of cell-adhesive receptors [5,6].

Integrins are heterodimeric membrane proteins composed of non-covalently associated α and β subunits. In mammals, 24 kinds of integrin heterodimers consisting of different α and β subunits have been identified [7]. Among them, eight integrins including α5β1 are known to bind to RGD-containing ligands such as fibronectin [7]. Modification of collagen matrices with RGD peptides has been shown to enhance mesenchymal stem cell adhesion [5]. Furthermore, incorporation of fibronectin-derived GRGDS and PHSRN (synergistic recognition site for integrin α5β1 binding) into collagen matrices enhances the cell adhesion and migration of mouse NIH3T3 cells [6]. While conjugation of RGD-containing peptides is effective in facilitating the adhesion of cells expressing

Please cite this article as: R. Sato-Nishiuchi, et al., Recombinant laminin fragments endowed with collagen-binding activity: A tool for conferring laminin-like c..., Matrix Biol (2017), http://dx.doi.org/10.1016/j.matbio.2017.08.001

RGD-binding integrins to collagen-based scaffolds, conjugation of other cell-adhesive proteins or their fragments is expected to be useful for the fabrication of collagen-based biomaterials targeting cells expressing different integrin types. In this study, we attempted to use laminins, major cell-adhesive proteins in basement membranes, to confer RGD-independent cell-adhesive activity on collagen matrices.

Laminins consist of α, β, and γ chains, which assemble into cross-shaped heterotrimers. To date, five α chains (α1–α5), three β chains (β1–β3), and three γ chains (γ1–γ3) have been identified, combinations of which yield at least 15 isoforms with distinct subunit compositions [8]. The expression patterns of laminin isoforms differ among different tissue types and developmental stages, suggesting that individual laminin isoforms may have distinct functions [9].

The interactions of cells with laminins are mediated by multiple cell surface receptors, including integrins, syndecans, and α-dystroglycan. Among these receptors, integrins play crucial roles in cell adhesion to laminins, and α3β1, α6β1, α7β1, and α6β4 integrins function as the major laminin receptors on the cell surface [10]. Comprehensive analyses of the interactions of laminin isoforms with four laminin-binding integrins revealed that laminin isoforms differ significantly in their integrin-binding specificities and affinities [11,12]. The integrin-binding activities of laminins were shown to be fully reproduced by their E8 fragments, consisting of the C-terminal coiled-coil domains of the α, β, and γ chains with extension of three laminin globular domains, LG1–LG3, at the C-terminus of the α chain [13–16]. We attempted to confer the cell-adhesive activity of laminins on collagen matrices using laminin E8 fragments, particularly the E8 fragment of laminin-511.

Laminin-511, composed of laminin α5, β1, and γ1 subunits, can sustain long-term self-renewal of human embryonic stem (ES) cells [17,18]. Furthermore, the laminin-511 E8 fragment (designated LM511E8) supports robust adhesion and expansion of dissociated human ES and induced pluripotent stem (iPS) cells [19,20]. Both laminin-511 and LM511E8 bind to integrin α6β1 with high affinity [15,16]. Human pluripotent stem cells (hPSCs) including ES and iPS cells predominantly express integrin α6β1 [17,18]. hPSCs avidly adhere to LM511E8-coated substrates and proliferate in an integrin α6β1-dependent manner, but cannot proliferate on collagen-coated substrates [19,21]. Given the abundant expression of α6β1 integrin on hPSCs, we reasoned that hPSCs could be used as a probe to detect the LM511E8-like activity conferred on collagen scaffolds. LM511E8 does not bind to collagen directly. In this study, we employed the collagen-binding domain (CBD) of fibronectin as a vehicle to confer the collagen-binding activity on LM511E8, with the aim of fabricating collagen-based scaffolds having laminin-like cell-adhesive activity.

Results

Production of collagen-binding LM511E8 fragments

We utilized the CBD of fibronectin to endow collagen-binding activity on LM511E8. Fibronectin is a multidomain protein composed of 12 FN-I modules, two FN-II modules, and 15–17 FN-III modules. The region from the 6th FN-I module to 9th FN-I module is known to bind to collagen (Fig. 1a) [22]. In vitro surveys of the abilities of different collagen types to bind to fibronectin revealed that the CBD of fibronectin binds to type I, type II, type III, and type IV collagens [23]. We produced LM511E8 fragments having the CBD of human fibronectin at the N-termini of the α5E8, β1E8, and γ1E8 chains. Through various combinations of the expression vectors for α5E8, β1E8, and γ1E8 with or without CBD, we produced seven CBD-attached LM511E8 fragments: LM511E8s having one CBD on α5E8 [CBD-LM511E8 (α)], β1E8 [CBD-LM511E8 (β)], or γ1E8 [CBD-LM511E8 (γ)], LM511E8s having two CBDs on α5E8/β1E8 [CBD-LM511E8 (αβ)], α5E8/γ1E8 [CBD-LM511E8 (αγ)], or β1E8/γ1E8 [CBD-LM511E8 (βγ)], and LM511E8 having three CBDs on all chains [CBD-LM511E8 (αβγ)] (Fig. 1b). The recombinant CBD-LM511E8s were expressed in 293-F cells and purified from the conditioned media by affinity chromatography using Ni-NTA agarose and anti-FLAG monoclonal antibody (mAb) M2-conjugated agarose columns.

The purified recombinant LM511E8 gave two bands migrating at 100 kDa and 75 kDa on SDS-PAGE under non-reducing conditions, corresponding to α5E8 and β1E8-γ1E8 dimer, respectively [16]. Likewise, CBD-LM511E8s gave two major bands, corresponding to α5E8 with or without CBD and β1E8-γ1E8 dimer with or without CBD on β1E8 or γ1E8 (Fig. 1c). These findings indicated that β1E8 and γ1E8 after CBD conjugation were competent in heterodimerization and subsequent assembly with α5E8 after CBD conjugation. The molecular masses were predicted to increase by ~40 kDa after attachment of one CBD. Therefore, the 140-kDa bands in CBD-LM511E8 (α), CBD-LM511E8 (αβ), CBD-LM511E8 (αγ), and CBD-LM511E8 (αβγ) corresponded to CBDα5E8, the 115-kDa bands in CBD-LM511E8 (β), CBD-LM511E8 (γ), CBD-LM511E8 (αβ), and CBD-LM511E8 (αγ) corresponded to CBDβ1E8-γ1E8 or β1E8-CBDγ1E8 dimer, and the 155-kDa bands in CBD-LM511E8 (βγ) and CBD-LM511E8 (αβγ) corresponded to CBDβ1E8-CBDγ1E8. The assignment of individual bands was confirmed by western blotting (Supplemental Fig. 1). The approximate yields of control LM511E8 and CBD-LM511E8s having one CBD were almost the same, while the yields of CBD-LM511E8s having two or three CBDs were

Please cite this article as: R. Sato-Nishiuchi, et al., Recombinant laminin fragments endowed with collagen-binding activity: A tool for conferring laminin-like c..., Matrix Biol (2017), http://dx.doi.org/10.1016/j.matbio.2017.08.001