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REVIEW ARTICLE

Heparanase Inhibitors Facilitate the Assembly of the Basement Membrane in Artificial Skin

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Abstract: Recent research suggests that the basement membrane at the dermal-epidermal junction of the skin plays an important role in maintaining a healthy epidermis and dermis, and repeated damage to the skin can destabilize the skin and accelerate the aging process. Skin-equivalent models are suitable for studying the reconstruction of the basement membrane and its contribution to epidermal homeostasis because they lack the basement membrane and show abnormal expression of epidermal differentiation markers. By using these models, it has been shown that reconstruction of the basement membrane is enhanced not only by supplying basement membrane components, but also by inhibiting proteinases such as urokinase and matrix metalloproteinase. Although matrix metalloproteinase inhibitors assist in the reconstruction of the basement membrane structure, their action is not sufficient to promote its functional recovery. However, heparanase inhibitors stabilize the heparan sulfate chains of perlecan (a heparan sulfate proteoglycan) and promote the regulation of heparan sulfate binding growth factors in the basement membrane. Heparan sulfate promotes effective protein-protein interactions, thereby facilitating the assembly of type VII collagen anchoring fibrils and elastin-associated microfibrils. Using both matrix metalloproteinase inhibitors and heparanase inhibitors, the basement membrane in a skin-equivalent model comes close to recapitulating the structure and function of an *in vivo* basement membrane. Therefore, by using an appropriate dermis model and suitable protease inhibitors, it may be possible to produce skin-equivalent models that are more similar to natural skin.

Keywords: Basement membrane, heparan sulfate, heparanase, inhibitor, matrix metalloproteinase, three-dimensional culture.

INTRODUCTION

The skin is composed of three distinct layers: the subcutaneous tissue, the dermis, and the epidermis. The epidermis is the uppermost or epithelial layer of the skin. It contains several cell types, including keratinocytes, melanocytes, Langerhans cells, and Merkel cells. The dermis lies between the epidermis and subcutaneous tissue and is mainly composed of fibroblasts, but also contains other cell types such as blood vessel cells, mast cells, and immune cells. The subcutaneous tissue is the fat layer below the dermis, and this contains cell types such as adipocytes, neurons, and blood vessel cells. Groups of cells within organs are surrounded by an extracellular matrix (ECM). The ECM provides the mechanical framework for each tissue and organ, is a substrate for cell signaling, supports organogenesis, and helps to maintain homeostasis [1]. In the skin, there are various types of extracellular matrix proteins, including collagens, elastins, and proteoglycans. Collagens are the most abundant component of the extracellular matrix. The dermis, for example, mainly contains type I and type III collagens, but also includes types V, VI, XII, and XIV [2].

For researchers seeking to understand cell proliferation and differentiation in the skin, one approach is to use an *in vitro* cell culture system that simulates the biochemical and mechanical signals between the dermis and epidermis mediated by the basement membrane (BM), thereby regulating tissue development [3]. However, the basement membrane at the interface of the dermis and epidermis is a critical component of a skin model that has been very difficult to recreate. Approaches to creating a model of the epidermal and dermal layers with an active basement membrane is the focus of this review.

SKIN-EQUIVALENT TISSUE ENGINEERING

In an *in vitro* tissue engineering system, cells are usually seeded onto three-dimensional polymer scaffolds that require

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an ECM composition suited for the attachment and growth of keratinocytes and promotes physical stability. An *in vitro* reconstructed skin model composed of the epidermal and dermal layers has been used as a model of skin constitution. Therefore, in these models, the layers are composed of skin fibroblasts, extracellular matrix, and keratinocytes. Bell *et al.* reported that a contracted collagen lattice containing fibroblasts might serve as a substrate for epidermal cells (Bell model) [4] and also presented a functional full-thickness skin model designed as a graft to cover open skin wounds or burned skin. In this dermal model, the proliferation of fibroblasts is inhibited similarly to the *in vivo* condition, and the fibroblasts are restricted in an environment of retracted collagen [5]. The model has been useful for exploring the dynamics of the BM [6, 7] and for studies of epidermal differentiation, dermal–epidermal interaction, and tumor cell invasion [8, 9]. However, its use is limited because a reduction in the final surface area of the skin equivalent (SE) can take place without a peripheral anchorage [10]. Skin model equivalents have been developed using many other methods that will not be covered here in detail (Table **1**). These models are distinguished from each other by how the dermal layer is created. The quality of the dermal equivalent should allow for the development and maintenance of a pluristratified and differentiated epidermis firmly anchored by an organized dermal-epidermal junction [11].

Keratinocytes are seeded onto the dermal layer and then cultured at the air-liquid interface, a condition that induces epidermal cell differentiation. Type I and III collagens are present in the reconstructed dermis, whereas type IV and VII collagens, as well as laminin, are deposited at the dermal-epidermal junction [12]. Conversely, epidermal quality may influence the properties of cells in the dermis [13]. For example, crosstalk between the epidermis and the dermis plays an important role in maintaining homeostasis of the skin [11, 14]. In addition to keratinocytes and fibroblasts, it is thought that melanocytes, vascular endothelial cells, neurons, and Langerhans cells in the skin might also be under the influence of such crosstalk [11, 15-19]. Therefore, the BM at the interface of the epidermis and dermis is critical for proper skin function [20].

BASEMENT MEMBRANE

The epidermal BM at the dermal-epidermal junction (DEJ) is mainly composed of type IV and Type VII collagens, several laminins (such as 332, 331, and 511), nidogen, and perlecan [21]. The anchoring fibrils, which anchor the BM to the underlying dermis, are composed primarily of type VII collagen [22]. The BM also contains a lamina densa (a sheet-like structure mainly composed of type IV collagen [23]) and a lamina lucida (the region between the lamina densa and the basal surface of the basal keratinocytes). It forms electron-dense plaques known as hemidesmosomes that contain at least two transmembrane proteins, α6β4 integrin and collagen type XVII (BP180). Hemidesmosomes link the epidermis to the BM [24, 25]. Perlecan, a heparan sulfate proteoglycan (HSPG) with three heparan sulfate chains, is a structural constituent of the BM at the DEJ [26]. The epidermis is structurally joined to the BM through hemidesmosomes and collagen type VII anchoring fibrils.

Disruption of the BM results in skin fragility and skin diseases such as epidermolysis bullosa [27]. Past studies have shown that gene abnormalities related to laminin 332, α6β4 integrin, BP180, and collagen type VII may be the cause of such issues [28-32] and they have highlighted the importance of the structural integrity of the interface between the epidermis and the dermis. Iriyama *et al.* reported that the heparan sulfate chains of perlecan were degraded by active heparanase in UVB-irradiated human skin and SEs, causing skin damage consistent with photoaging [33].

Proteinases such as metalloproteinases and the plasmin-plasminogen system are involved in normal and aberrant cutaneous wound repair. Some proteases also activate growth factors and other proteases in wounds, and regulate growth factor signaling by shedding growth factor receptors on the cell surface. Furthermore, proteases break down the

Table 1. Various methods of dermal model creation.

	Dermal equivalent			Culture period	Ref.
	Polymer scaffolds	Cell Types	Additives		
1	Animal collagen I	fibroblast	no	3~ 4days	[4]
2	Collagen sponge Fibrin matrix	fibroblast	no	4 weeks	[133-135]
3	Esterified hyaluronic acid fibers Fibrin matrix	fibroblast	TGF-beta	6 weeks	[136]
4	Matrix complex (type I and III collagens and chondroitin sulfate)	fibroblast	no	3 weeks	[137, 138]
5	No exogenous scaffolding elements	fibroblast	no	4 weeks	[139-141]
6	Fibronectin Collagen	fibroblast huvecs	no	7 days	[142]
7	De-epidermized derma	-	no	-	[143]

ECM produced by cells [34], an effect that should be recapitulated in a skin model. Therefore, in this review, I discuss how the combined use of protease inhibitors in a skin model culture can affect the reconstruction of the BM and the function of a skin model.

THE ADHESION OF THE EPIDERMIS AND THE DERMIS IN SEs

To generate a typical SE model, human keratinocytes are seeded onto a contracted collagen gel containing dermal fibroblasts, and specific culture conditions are used to ensure that the model is highly similar to native tissue [35]. These models are an important tool for the study of cell-cell, cell-matrix, and dermal-epidermal interactions, and for pharmacotoxicological testing. However, currently, human SEs have a limited lifespan of approximately eight weeks, rendering them unsuitable for long-term studies. Further, the structural integrity of the interface joining the dermis and epidermis is fragile. Therefore, studies requiring a skin model are typically based on non-human dermal equivalents, *e.g*., collagen originating from rat tendons, bovine tissues, and pigs' skin. Therefore, these alternative non-human biopolymers allow for the rapid formation of SEs [36].

De-epidermized dermis (DED, Table **1**) may offer a human alternative to animal derived dermal equivalents, but the application of this method is limited by uncontrollable variation in DED thickness and low availability of the native tissue. Other dermal equivalents (Table **1**) can be generated using a primary human fibroblast-derived dermal matrix with, for example, chitosan-cross-linked collagen-glycosaminoglycan. In these methods, cultivation must be maintained for weeks in order to form a dermal model. Keratinocytes are then seeded onto the dermal model, leading to the formation of the epidermis structure (this process is common to all methods), which takes a week. The most important factor for success is a strong connection between the dermis and the epidermis through the BM, similar to that in the living body. Additionally, the communication between the formed epidermal and dermal layers should be normal and continually maintained. Dynamic interactions and the cooperation of many components, from fibroblasts to keratinocytes, are required to create a skin-specific ECM, and the BM dermo-epidermal junction is vital. The BM has been reported to play an important role in the attachment of the epidermis, the regulation of cellular polarity, and the survival, proliferation, and differentiation of cells [37-40].

Type I, III, and V collagens and fibrillin-1 are derived from fibroblasts, whereas the components of the hemidesmosome, BP180 and integrin β4, are derived from keratinocytes [41]. However, tenascin-C, type IV and VII collagens, perlecan, and nidogen are synthesized by both fibroblasts and keratinocytes. Many components produced by both fibroblasts and keratinocytes are required for the formation of a normal epidermis and BM in SEs, and factors secreted by keratinocytes activate matrix metalloproteinases (MMPs).

Previous studies have demonstrated that treatment with the synthetic MMP inhibitor CGS27023, which blocks degradation of type IV collagen, leads to the formation of a continuous lamina densa in SEs [42]. This suggests that a collagen degradation system is active in SE models. In previously described three-dimensional skin models, the lamina densa and hemidesmosomes were visible in some areas after two weeks, and anchoring fibrils were organized after six weeks of culture [43]. Marionnet *et al*. detected expression of BM components after just a few days of culture, and immunostaining revealed that BM proteins were at the DEJ after eight days of culture [44]. These reports suggest that long cultivation times are needed to form the basement membrane, even if the expression and deposition of basement membrane components were detected early. In other words, in SE models that lack a BM at the early stage of culture, a balance in the production and degradation of matrix proteins affects protein assembly and the higher-order structure of the BM.

UVB exposure to skin is known to increase the synthesis of MMPs, urokinase-type plasminogen activator (uPA), uPAR, and heparanase, which degrades BM components such as type VII and IV collagens, laminin 332, and perlecan [33, 45-48]. SE models can partially mimic the photo-aging process because BMs are missing at the DEJ and large amounts of MMP (including gelatinases MMP-2 and MMP-9), active uPA, and uPA are present in the conditioned SE media [7, 42].

Additionally, a plasmin inhibitor, aprotinin, can restore the assembly of the BM at the DEJ following damage caused by the addition of plasminogen [48]. Laminin 332 is also capable of enhancing BM assembly [6]. Battaglia *et al.* showed that HSPG promoted assembly of the BM by interacting with components of the BM, such as laminins [49]. Furthermore, the deposition of collagen VII is polarized to the basal side by the addition of an MMP inhibitor, and treatment with a combination of MMP inhibitor and heparanase inhibitor enhances the formation of anchoring fibrils that bind to the lamina densa. Sher *et al*. reported that keratinocyte-derived cell perlecan is essential for the survival of the regenerative cell population required to form a multilayered epidermis [50]. Together these findings suggest that if a uniform lamina densa with anchoring fibrils that bind to the basal lamina is formed, a typical SE can be produced that firmly connects dermis, epidermis, and BM.

THE ROLE OF HEPARAN SULFATE IN ELASTIC FIBER FORMATION IN THE SE

Elastic fibers, which consist of polymerized tropoelastin monomers surrounded by a mantle of microfibrils (MFs) (reviewed in [51]), undergo normal age-related changes, leading to loose skin, stiff vessels, and increased blood pressure. In living skin, mature elastin fibrils are distributed among collagen bundles. In electron micrographs, elastic fibers appear as an amorphous core of elastin surrounded by 12-nm diameter MFs containing the glycoprotein fibrillin. The primary roles of elastic fibers include providing elasticity and recoil in tissues and organs and maintaining structural integrity against mechanical strain over a lifetime [52]. Moreover, elastic fibers, such as oxytalan fibers in papillary dermis, are associated with not only skin resilience, but also skin surface texture [53, 54]. Yamazaki *et al.* reported that elastin-free oxytalan MFs originating from the DEJ are continuous with elaunin-type MFs (with a small amount of

elastin) in the deeper papillary dermis, whereas the reticular dermis contains elastic fibers or MFs embedded largely in elastin [55]. It has been suggested that aggregates of MFs originating from the DEJ are important for oxytalan fiber formation because MFs are thought to facilitate alignment of elastin monomers prior to cross-linking by lysyl oxidase (LOX) [56, 57].

Casasco *et al*. studied elastin expression using a SE with a dermis model made of a collagen gel [58]. They reported that, after about two weeks of culture, high-resolution immunocytochemistry and electron microscopy could be used for specific labeling and detection of the elastin-immunoreactive material in the ECM. However, the distribution of immunoreactive material was small and it was not assembled into fibers. They also reported that a well-developed rough endoplasmic reticulum was visible in the fibroblasts of the dermal model. Duplan-Perrat *et al*. also investigated elastin formation in a dermal model made of a sponge made of collagen, chitosan, and GAGs. The expression of elastin in the SE was demonstrated 30 days after culture (15 days after the seeding keratinocytes). Using electron microscopy, elastin could be seen only after 45 days (30 days after keratinocyte-seeding). Clearly, the formation of elastin requires long time periods [53]. The authors suggested that MFs first assembled at the DEJ without elastin, and then elastin was deposited on the hollow-shaped MF bundles beneath the DEJ, and finally, true elastic fibers, combining microfibrilar and amorphous components, formed [59]. It has also been reported that the maturation of elastic fibers is affected by keratinocytes [60]; the synthesis and assembly of fibrillin into MFs concomitant with basal lamina formation favored the regular perpendicular distribution of oxytalan fibers at the DEJ in SE models. It was also reported that the presence of keratinocytes and ECM generated by fibroblasts in the porous structures of the dermis were needed to trigger elastin deposition on MFs [61].

In natural skin, mature elastin fibers are distributed between mesh-like layers of collagen fibers. The main components of elastin fibers are amorphous elastin and MFs, but they also contain many other proteins such as tropoelastin, fibulin, fibrillin, nidogen, laminin, perlecan, and LTBP [62-65]. Several groups have reported that heparan sulfates are associated with elastin-related proteins [66-71]. Tiedemann *et al*. demonstrated that the proteoglycan perlecan is an associated with MFs in close proximity to BM zones and revealed interactions between perlecan domains I/II and a central region of fibrillin-1. They also reported that perlecan null embryos had fewer MFs at the DEJ when compared with wild-type littermates [72]. Ritty *et al*. reported that interactions between heparan sulfate and fibrillins play a critical role in fibrillin network assembly and are important for matrix deposition by fibroblasts [73]. Sabatieret *et al*. suggested that heparan sulfates could play a role in stabilizing the growth of MFs, and could assist in the process of elongation and interactions with other MF-associated proteins [71]. Based on these reports, the presence of perlecan with heparan sulfate is expected to play an important role in elastin formation. Iriyama *et al*. reported that when the heparanase inhibitor (1-[4-(1H-benzoimidazol-2-yl)-phenyl]-3-[4-(1H-benzoimidazol-2-yl)-phenyl]-urea (BIPBIPU) [74]) and a matrix metalloproteinase inhibitor were applied to a SE, regeneration and repair of the dermal and epidermal BM was significantly enhanced [75]. Additionally, a previous study showed that elastin-like structures similar to the elastin reported by Rodrigo *et al*. was observed in a skin model only after cultures were treated with both a heparanase inhibitor and MMP inhibitor (Fig. **1**) [76, 77].

Together, these results suggest that a more stable artificial skin model could be produced in relatively short-term culture if a suitable combination of inhibitors that influence the structure and composition of the extracellular matrix were identified and characterized.

HEPARANASE

Heparan sulfate specific endo-beta-D-glucuronidase activity in normal tissue was first reported by Höök *et al*. in 1975, and today the enzyme is known as heparanase [78, 79]. Heparanase is well studied in cancer research because it is a useful marker for tumor metastasis. The heparan sulfate side chains of the proteoglycan scaffold in the ECM can be digested by heparanase. The enzyme is implicated not only in

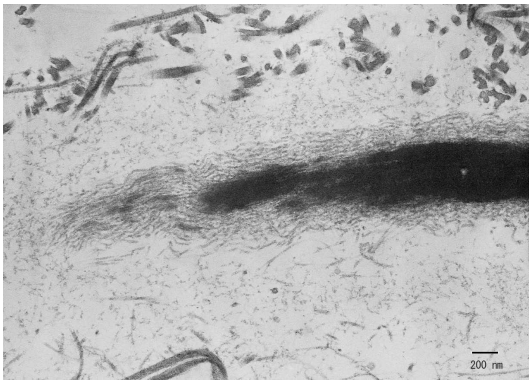

Fig. (1). A skin-equivalent was fixed using Zamboni's Fixative. The fixed sample was sectioned and stained with 0.5% uranyl acetate at 4 °C for 24 h. After the osmiun-postfixed sample was immersed in 0.5% tannic acid overnight, the pieces were embedded in epoxy resin. Elastic fibers are mainly composed of microfibrils and amorphous elastin. This electron microscopy image shows a region relatively deep in the dermis of a skin-equivalent following culture with protease inhibitors. Microfibrils assembled into an elastic-like structure were only observed when the artificial skin (Bell type) was cultured with a matrix metalloproteinase inhibitor (10^{-5} M; N-hydroxy-2-[[(4-methoxyphenyl) sulfonyl] 3-picolyl) amino]-3-methylbutanamide hydrochloride) and heparanase inhibitor (10^{-5} M; 1-[4-(1H-benzoimidazol-2-yl)-phenyl]-3-[4-(1H-benzoimidazol-2-yl)-phenyl]-urea). Scale bar: 200 nm.

tumor metastasis but also angiogenesis, inflammation, and autoimmunity [80]. Lerner *et al.* revealed involvement of heparanase in the pathogenesis of psoriasis and suggested a role for the enzyme in facilitating abnormal interactions between immune and epithelial cellular subsets of the affected skin [81]. It is also involved in extracellular matrix remodeling and degradation [20, 82]. Heparanase activity has been observed in fibroblasts, mast cells, platelets, placenta, smooth muscle cells, macrophages, monocytes, neutrophils, and Langerhans cells [83, 84]. Heparanase is expressed at the stratum corneum, epidermis, the inner root sheath of hair follicles in the skin, and the esophageal epithelium [85, 86]. It is thought to be involved in differentiation, wound healing, hair follicle homeostasis, and sweat gland morphogenesis [87-89].

Heparanase is synthesized as a latent 65-kDa protein and is processed in the lysosomal compartment to an active 58-kDa heterodimer, which consists of a 50-kDa protein and an 8-kDa peptide [90, 91]. Vlodavsky *et al*. reported that the active form is 100-fold more active than the 65-kDa form, which is highly active in acidic conditions (pH 4-6.5) [92, 93]. Heparanase cleaves heparan sulfate at specific intrachain sites involving beta-D-glucuronosyl-N-acetylglucosaminyl linkages [94]. The 65-kDa latent heparanase is processed by the lysosomal enzymes cathepsin D and L, which results in the activated dimer form. Reinheckel *et al.* showed that cathepsin L is expressed in keratinocytes specific to the skin [95]. Cathepsin L is associated with the inflammasome, and is reported to be involved in the activation of NLRP3. Riteau *et al*. reported that inflammasome activity is stimulated *via* the P2Y1 receptor [96]. Specifically, it was found that P2Y, a G-protein-coupled receptor, is activated by various nucleotides (adenosine, ATP, and ADP) [91, 97]. Additionally, Inoue *et al.* reported that large amounts of extracellular and total cellular ATP were observed in cells that were exposed to UVB radiation [98]. Heparanase in human keratinocytes is activated by UVB exposure, probably because of ATP release and P2Y signaling related to a damage signal [33]. Iriyama *et al*. showed also that repeated barrier-disruption of the skin leads to upregulation of heparanase, its activation in the epidermis [85], and to increased growth factor interactions between the epidermis and dermis, which facilitates various cutaneous changes, including wrinkle formation.

THE FUNCTION OF HSPGs IN THE BASEMENT MEMBRANE

Heparan sulfate is the most abundant of the GAG family, and is present through the epidermis [99, 100]. It is a linear polysaccharide chain composed of D-glucosamine, D-glucuronic acid, and L-iduronic acid [101]. The largest part of each GAG chain contains a number of repeating disaccharide units composed of alternating glucuronic acid and N-acetylglucosamine, which during biosynthesis are subjected to a range of modifications, including O-sulfation at various positions, N-deacetylation, N-sulfation of N-acetylglucosamine residues, and C-5 epimerization of glucuronic acid to iduronic acid. Theoretically, up to 48 different disaccharides could occur in heparan sulfate because of these modifications [102]. Therefore, it is possible that a large number of sequences exist, and such structural diversity underlies the diversified biological functions of GAGs.

HSPGs consist of a protein core to which heparan sulfate side chains are covalently attached. These complex macromolecules are highly abundant in the ECM and are thought to play an important structural role, contributing to ECM integrity and insolubility [103]. HSPGs are involved in the regulation of molecular transfer through the BM, protection of matrix components such as type IV collagen from proteolytic attack, storage of various cytokines and growth factors, and are key molecular players in inflammation [94, 104].

Heparanase contributes to angiogenesis because HSPGs are cleaved by heparanase, releasing angiogenic factors such as fibroblast growth factors (FGFs), vascular endothelial growth factors A (VEGF-A), heparin growth factors, platelet-derived growth factors, and transforming growth factor-β [93, 105-114]. Since heparan sulfate side chains produce a large negative charge, HSPGs create a diffusion barrier, as well as an anchoring port. For example, heparan sulfate regulates the activity of FGFs; heparan sulfate-binding growth factors, by acting as coreceptors on the cell surface, enhance FGF receptor affinity for FGF [110, 115], providing a storage reservoir in the BM [102, 108, 116-118]. Heparan sulfate also regulates angiogenesis *via* its interaction with VEGF-A. Kiryushko *et al.* showed that signaling through S100A4, part of the S100 family, depends on interactions with HSPGs at the cell surface [119]. Thus, GAGs may act as coreceptors for S100 proteins in neurons [119-121]. Furthermore, heparanase-induced loss of heparan sulfate at the DEJ has been implicated in the process of pigmentation in human skin. Specifically, proliferation and differentiation of melanocytes is regulated by various factors, including leukemia inhibitory factor (LIF), stem cell factor (SCF), basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), and granulocyte-macrophage colony stimulating factor (GM-CSF). Because HGF and bFGF bind to heparan sulfate at the DEJ, these growth factors are restricted from reaching melanocytes in normal conditions [20, 122-124]. Therefore, taken together, these studies show that HSPGs are essential for several important biological processes.

THE ROLE OF HSPGs IN DEJ ORGANIZATION IN THE SE

The von Willebrand factor domain, including the NC-1 domain of type VII collagen, interacts with heparin and proteoglycans [125]. Therefore, an increase in heparin sulfate chains in the presence of heparanase inhibitor promotes effective interaction between the NC-1 domain of type VII collagen and the beta 3 chain of laminin 332 at the DEJ, facilitating the assembly of anchoring fibrils. Iriyama *et al.* showed that the addition of a heparanase inhibitor, together with an MMP inhibitor, promoted anchoring fibril structure formation in the BM in a SE model [126]. Pineou *et al.* showed that synthesis of heparan sulfate proteoglycans such as syndecan and perlecan (a mixed heparan sulfate/chondroitin sulfate proteoglycan localized at the skin BM zone) was induced by a xylopyranoside derivative, which

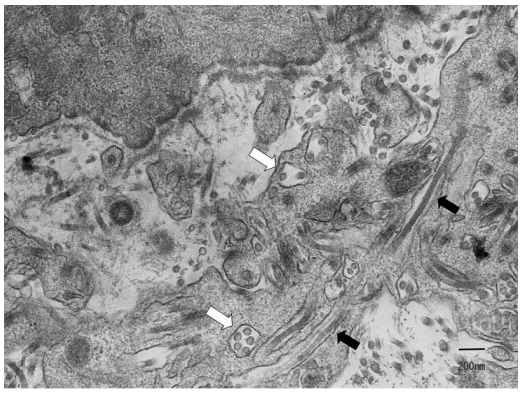

Fig. (2). Fibripositor formation in a skin-equivalent produced following culture with a matrix metalloproteinase inhibitor and a heparanase inhibitor. The electron microscopy image shows a region under the basement membrane at the dermal-epidermal junction in a skin-equivalent cultured with protease inhibitors. Part of a fibroblast is shown, as are plasma membrane collagens (white arrow). Collagen fibrils (black arrow) are continuous from the extracellular matrix through the lumen of the fibripositor. Scale bar: 200 nm.

also increased GAG synthesis. In addition, a xylopyranoside derivative increased the expression of BM components such as type IV and VII collagens, procollagen-1, laminin 332, and fibrillin-1 in human skin [127]. These reports suggest that the increase in HSPGs impacts DEJ organization by decomposition, suppression, or synthetic induction of heparan sulfate side chains.

Sahuc *et al.* used electron microscopy to show that linear deposition of type VII collagen was detected in immunohistochemical studies after two weeks of culture, and anchoring fibrils, a continuous lamina densa, and numerous hemidesmosomes were detected where keratinocytes were in direct contact with fibroblasts [128]. Breitkreutz *et al.* observed BM formation during skin regeneration in human keratinocyte transplants in nude mice. They showed that the deposition of type VII collagen became continuous and BM-anchoring fibrils developed at 22 days, indicating final tissue consolidation [129]. Although the formation of the basement membrane takes time in the living body, the use of appropriate enzyme inhibitors and/or protein production enhancers, such as MMP inhibitors, heparanase inhibitors, and GAG synthesis inducers could lead to the faster deposition of BM components and assist in the maturation of BM structure. By using heparanase and MMP inhibitors, it was possible to create a typical SE that closely resembles the structure of natural skin in a relatively short period (~4-14 days) [75].

In addition to accelerating the maturation of BMs, heparanase and MMP inhibitors also indicate that presence of active fibroblasts in the dermis of artificial skin. In SEs, similar to natural skin, collagen synthesis, protein secretion, and cellular production of ECM and fibers is active. Moreover, in the dermis of SEs cultivated using MMP inhibitors and heparanase inhibitors, the formation of higher-order structures of fibroblasts, including fibripositors and rough endoplasmic reticulum, have been observed (Fig. **2**). Fibripositors are actin-rich plasma membrane protrusions that secrete and deposit collagen fibrils. Moreover, this phenomenon has only been observed with embryonic fibroblasts [130-132]. Fibroblasts with fibripositors are considered active and have been observed beneath the BM, close to the epidermis. However, the mechanism underlying the formation of fibripositors and collagen secretion from fibroblasts, which is induced by MMP and heparanase inhibitors, is currently unknown. Further studies are needed to understand the mechanisms controlling dermal tissue formation and the influence of the epidermis and basement membrane.

SUMMARY

One approach to understanding the mechanism of cell proliferation and differentiation is the *in vitro* study of three-dimensional cell cultures that simulate the biochemical and mechanical signals that regulate tissue development. The functional BM at the interface of the epidermis and the dermis is important for epidermal differentiation. In addition to type IV and VII collagens, which are important for the formation of BMs, the heparan sulfate chains of proteoglycans such as perlecan give functionality to the BM. The use of appropriate enzyme inhibitors and/or protein production enhancers could promote BM production and speed wound healing in living tissue. Additionally, the artificial skin model is a useful tool for investigating the crosstalk that exists between the epidermis and the dermis.

CONFLICT OF INTEREST

The author confirms that this article content has no conflict of interest.

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REFERENCES

[1] Brown BN, Badylak SF. Extracellular matrix as an inductive scaffold for functional tissue reconstruction. Transl Res 2014; 163(4): 268-85.

[2] van der Rest M, Garrone R. Collagen family of proteins. FASEB J 1991; 5(13): 2814-23.

[3] Freed LE, Gordana V-N. In: Lanza RP, Langer R, Vacanti J, Ed. Principles of Tissue Engineering. 2nd ed. San Diego, Academic Press 2000; pp. 143-56.

[4] Bell E, Ivarsson B, Merrill C. Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential *in vitro*. Proc Natl Acad Sci USA 1979; 76(3): 1274-8.

[5] Berthod F, Hayek D, Damour O, Collombel C. Collagen synthesis by fibroblasts cultured within a collagen sponge. Biomaterials 1993; 14(10): 749-54.

[6] Tsunenaga M, Adachi E, Amano S, Burgeson RE, Nishiyama T. Laminin 5 can promote assembly of the lamina densa in the skin equivalent model. Matrix Biol 1998; 17(8-9): 603-13.

[7] Amano S, Akutsu N, Matsunaga Y, *et al.* Importance of balance between extracellular matrix synthesis and degradation in basement membrane formation. Exp Cell Res 2001; 271(2): 249-62.

[8] Tsunenaga M, Kohno Y, Horii I, *et al.* Growth and differentiation properties of normal and transformed human keratinocytes in organotypic culture. Jpn J Cancer Res 1994; 85(3): 238-44.

[9] Nishiyama T, Amano S, Tsunenaga M, *et al.* The importance of laminin 5 in the dermal-epidermal basement membrane. J Dermatol Sc 2000; 24 Suppl 1: S51-9.

[10] Lopez Valle CA, Auger FA, Rompre P, Bouvard V, Germain L. Peripheral anchorage of dermal equivalents. Br J Dermatol 1992; 127(4): 365-71.

[11] Auxenfans C, Fradette J, Lequeux C, *et al.* Evolution of three dimensional skin equivalent models reconstructed *in vitro* by tissue engineering. Eur J Dermatol 2009; 19(2): 107-13.

[12] Laplante AF, Germain L, Auger FA, Moulin V. Mechanisms of wound reepithelialization: hints from a tissue-engineered reconstructed skin to long-standing questions. FASEB J 2001; 15(13): 2377-89.

[13] Bellemare J, Roberge CJ, Bergeron D, Lopez-Valle CA, Roy M, Moulin VJ. Epidermis promotes dermal fibrosis: role in the pathogenesis of hypertrophic scars. J Pathol 2005; 206(1): 1-8.

[14] Duval C, Chagnoleau C, Pouradier F, Sextius P, Condom E, Bernerd F. Human skin model containing melanocytes: essential role of keratinocyte growth factor for constitutive pigmentation-functional response to alpha-melanocyte stimulating hormone and forskolin. Tissue Eng Part C Methods 2012; 18(12): 947-57.

[15] Topol BM, Haimes HB, Dubertret L, Bell E. Transfer of melanosomes in a skin equivalent model *in vitro*. J Invest Dermatol 1986; 87(5): 642-7.

[16] Okazaki M, Suzuki Y, Yoshimura K, Harii K. Construction of pigmented skin equivalent and its application to the study of congenital disorders of pigmentation. Scand J Plast Reconstr Surg Hand Surg 2005; 39(6): 339-43.

[17] Hudon V, Berthod F, Black AF, Damour O, Germain L, Auger FA. A tissue-engineered endothelialized dermis to study the modulation of angiogenic and angiostatic molecules on capillary-like tube formation *in vitro*. Br J Dermatol 2003; 148(6): 1094-104.

[18] Laubach V, Zoller N, Rossberg M, *et al.* Integration of Langerhans-like cells into a human skin equivalent. Arch Dermatol Res 2011; 303(2):135-9.

[19] Bechetoille N, Dezutter-Dambuyant C, Damour O, Andre V, Orly I, Perrier E. Effects of solar ultraviolet radiation on engineered human skin equivalent containing both Langerhans cells and dermal dendritic cells. Tissue Eng 2007; 13(11): 2667-79.

[20] Iriyama S, Ono T, Aoki H, Amano S. Hyperpigmentation in human solar lentigo is promoted by heparanase-induced loss of heparan sulfate chains at the dermal-epidermal junction. J Dermatol Sci 2011; 64(3): 223-8.

[21] Marinkovich MP, Keene DR, Rimberg CS, RE. B. Cellular origin of the dermal-epidermal basement membrane. Dev Dyn 1993; 197: 255-67.

[22] Sakai LY, Keene DR, Morris NP, Burgeson RE. Type VII collagen is a major structural component of anchoring fibrils. J Cell Biol 1986; 103: 1577-86.

[23] Adachi E, Hopkinson I, Hayashi T. Basement-membrane stromal relationships: interactions between collagen fibrils and the lamina densa. Int Rev Cytol 1997; 173: 73-156.

[24] Stepp MA, Spurr-Michaud S, Tisdale A, Elwell J, Gipson IK. Alpha 6 beta 4 integrin heterodimer is a component of hemidesmosomes. Proc Natl Acad Sci USA 1990; 87(22): 8970-4.

[25] Diaz LA, Ratrie H, 3rd, Saunders WS, *et al.* Isolation of a human epidermal cDNA corresponding to the 180-kD autoantigen recognized by bullous pemphigoid and herpes gestationis sera. Immunolocalization of this protein to the hemidesmosome. J Clin Invest 1990; 86(4): 1088-94.

[26] Prunieras M, Regnier M, Fougere S, Woodley D. Keratinocytes synthesize basal-lamina proteins in culture. J Invest Dermatol 1983; 81(1 Suppl): 74s-81s.

[27] Niessen CM, van der Raaij-Helmer MH, Hulsman EH, van der Neut R, Jonkman MF, Sonnenberg A. Deficiency of the integrin beta 4 subunit in junctional epidermolysis bullosa with pyloric atresia: consequences for hemidesmosome formation and adhesion properties. J Cell Sci 1996; 109 (Pt 7): 1695-706.

[28] Vailly J, Pulkkinen L, Miquel C, *et al.* Identification of a homozygous one-basepair deletion in exon 14 of the LAMB3 gene in a patient with Herlitz junctional epidermolysis bullosa and prenatal diagnosis in a family at risk for recurrence. J Invest Dermatol 1995; 104(4): 462-6.

[29] Ruzzi L, Gagnoux-Palacios L, Pinola M, *et al.* A homozygous mutation in the integrin alpha6 gene in junctional epidermolysis bullosa with pyloric atresia. J Clin Invest 1997; 99(12): 2826-31.

[30] Huber M, Floeth M, Borradori L, *et al.* Deletion of the cytoplasmatic domain of BP180/collagen XVII causes a phenotype with predominant features of epidermolysis bullosa simplex. J Invest Dermatol 2002; 118(1): 185-92.

[31] Ito K, Sawamura D, Goto M, *et al.* Keratinocyte-/fibroblast-targeted rescue of Col7a1-disrupted mice and generation of an exact dystrophic epidermolysis bullosa model using a human COL7A1 mutation. Am J Pathol 2009; 175(6): 2508-17.

[32] Laimer M, Lanschuetzer CM, Diem A, Bauer JW. Herlitz junctional epidermolysis bullosa. Dermatol Clin 2010; 28(1): 55-60.

[33] Iriyama S, Matsunaga Y, Takahashi K, Matsuzaki K, Kumagai N, Amano S. Activation of heparanase by ultraviolet B irradiation leads to functional loss of basement membrane at the dermal-epidermal junction in human skin. Arch Dermatol Res 2011; 303(4): 253-61.

[34] Toriseva M, Kahari VM. Proteinases in cutaneous wound healing. Cell Mol Life Sci 2009; 66(2): 203-24.

[35] Bell E, Sher S, Hull B, *et al.* The reconstitution of living skin. J Invest Dermatol 1983; 81(1 Suppl): 2s-10s.

[36] El Ghalbzouri A, Commandeur S, Rietveld MH, Mulder AA, Willemze R. Replacement of animal-derived collagen matrix by human fibroblast-derived dermal matrix for human skin equivalent products. Biomaterials 2009; 30(1): 71-8.

[37] Ogura Y, Matsunaga Y, Nishiyama T, Amano S. Plasmin induces degradation and dysfunction of laminin 332 (laminin 5) and impaired assembly of basement membrane at the dermal-epidermal junction. Br J Dermatol 2008; 159(1): 49-60.

[38] Lechler T, Fuchs E. Asymmetric cell divisions promote stratification and differentiation of mammalian skin. Nature 2005; 437(7056): 275-80.

[39] Boudreau N, Werb Z, Bissell MJ. Suppression of apoptosis by basement membrane requires three-dimensional tissue organization and withdrawal from the cell cycle. Proc Natl Acad Sci 1996; 93(8): 3509-13.

[40] Segal N, Andriani F, Pfeiffer L, *et al.* The basement membrane microenvironment directs the normalization and survival of bioengineered human skin equivalents. Matrix Bio 2008; 27(3): 163-70.

[41] O'Toole EA. Extracellular matrix and keratinocyte migration. Clin Exp Dermatol 2001; 26(6): 525-30.

[42] Amano S, Ogura Y, Akutsu N, *et al.* Protective effect of matrix metalloproteinase inhibitors against epidermal basement membrane damage: skin equivalents partially mimic photoageing process. Br J Dermatol 2005; 153 Suppl 2: 37-46.

[43] Stark HJ, Boehnke K, Mirancea N, *et al.* Epidermal homeostasis in long-term scaffold-enforced skin equivalents. J Investig Dermatol Symp Proc 2006; 11(1): 93-105.

[44] Marionnet C, Pierrard C, Vioux-Chagnoleau C, Sok J, Asselineau D, Bernerd F. Interactions between fibroblasts and keratinocytes in morphogenesis of dermal epidermal junction in a model of reconstructed skin. J Invest Dermatol 2006; 126(5): 971-9.

[45] Fisher GJ, Datta SC, Talwar HS, *et al.* Molecular basis of sun-induced premature skin ageing and retinoid antagonism. Nature 1996; 379(6563): 335-9.

[46] Miralles F, Parra M, Caelles C, Nagamine Y, Felez J, Munoz-Canoves P. UV irradiation induces the murine urokinase-type plasminogen activator gene *via* the c-Jun N-terminal kinase signaling pathway: requirement of an AP1 enhancer element. Mol Cell Biol 1998; 18(8): 4537-47.

[47] Marschall C, Lengyel E, Nobutoh T, *et al.* UVB increases urokinase-type plasminogen activator receptor (uPAR) expression. J Invest Dermatol 1999; 113(1): 69-76.

[48] Amano S. Possible involvement of basement membrane damage in skin photoaging. J Investig Dermatol Symp Proc 2009; 14(1): 2-7.

[49] Battaglia C, Mayer U, Aumailley M, Timpl R. Basement-membrane heparan sulfate proteoglycan binds to laminin by its heparan sulfate chains and to nidogen by sites in the protein core. Eur J Biochem 1992; 208: 359-66.

[50] Sher I, Zisman-Rozen S, Eliahu L, *et al.* Targeting perlecan in human keratinocytes reveals novel roles for perlecan in epidermal formation. J Biol Chem 2006; 281(8): 5178-87.

[51] Wagenseil JE, Mecham RP. New insights into elastic fiber assembly. Birth Defects Res C Embryo Today 2007; 81(4): 229-40.

[52] Yanagisawa H, Davis EC. Unraveling the mechanism of elastic fiber assembly: The roles of short fibulins. Int J Biochem Cell Biol 2010; 42(7): 1084-93.

[53] Kadoya K, Amano S, Inomata S, *et al.* Evaluation of autologous cultured epithelium as replacement skin after tattoo excision: correlation between skin texture and histological features. Br J Dermatol 2003; 149(2): 377-80.

[54] Kadoya K, Amano S, Nishiyama T, *et al.* Changes in fibrillin-1 expression, elastin expression and skin surface texture at sites of cultured epithelial autograft transplantation onto wounds from burn scar excision. Int Wound J 2015: http://dx.doi.org/10.1111/iwj.12375.

[55] Yamazaki Y, Sejima H, Yuguchi M, Namba Y, Isokawa K. Late deposition of elastin to vertical microfibrillar fibers in the presumptive dermis of the chick embryonic tarsometatarsus. Anat Rec (Hoboken) 2007; 290(10): 1300-8.

[56] Smith-Mungo LI, Kagan HM. Lysyl oxidase: properties, regulation and multiple functions in biology. Matrix Biol 1998; 16(7): 387-98.

[57] Bouez C, Reynaud C, Noblesse E, *et al.* The lysyl oxidase LOX is absent in basal and squamous cell carcinomas and its knockdown induces an invading phenotype in a skin equivalent model. Clin Cancer Res 2006; 12(5): 1463-9.

[58] Casasco M, Casasco A, Icaro Cornaglia A, Farina A, Calligaro A. Differential distribution of elastic tissue in human natural skin and tissue-engineered skin. J Mol Histol 2004; 35(4): 421-8.

[59] Duplan-Perrat F, Damour O, Montrocher C, *et al.* Keratinocytes influence the maturation and organization of the elastin network in a skin equivalent. J Invest Dermatol 2000; 114(2): 365-70.

[60] Raghunath M, Bachi T, Meuli M, *et al.* Fibrillin and elastin expression in skin regenerating from cultured keratinocyte autografts: morphogenesis of microfibrils begins at the dermo-epidermal junction and precedes elastic fiber formation. J Invest Dermatol 1996; 106(5): 1090-5.

[61] Noblesse E, Cenizo V, Bouez C, *et al.* Lysyl oxidase-like and lysyl oxidase are present in the dermis and epidermis of a skin equivalent and in human skin and are associated to elastic fibers. J Invest Dermatol 2004; 122(3): 621-30.

[62] Sasaki T, Gohring W, Miosge N, Abrams WR, Rosenbloom J, Timpl R. Tropoelastin binding to fibulins, nidogen-2 and other extracellular matrix proteins. FEBS Lett 1999; 460(2): 280-4.

[63] Timpl R, Sasaki T, Kostka G, Chu ML. Fibulins: a versatile family of extracellular matrix proteins. Nat. Rev. Mol. Cell Biol 2003; 4(6): 479-89.

[64] Zheng Q, Davis EC, Richardson JA, *et al.* Molecular analysis of fibulin-5 function during de novo synthesis of elastic fibers. Mol Cell Biol 2007; 27(3): 1083-95.

[65] Sideek MA, Menz C, Parsi MK, Gibson MA. LTBP-2 competes with tropoelastin for binding to fibulin-5 and heparin, and is a negative modulator of elastinogenesis. Matrix Biol 2014; 34: 114-23.

[66] Hopf M, Gohring W, Mann K, Timpl R. Mapping of binding sites for nidogens, fibulin-2, fibronectin and heparin to different IG modules of perlecan. J Mol Biol 2001; 311(3): 529-41.

[67] Hayes AJ, Lord MS, Smith SM, *et al.* Colocalization *in vivo* and association *in vitro* of perlecan and elastin. Histochem Cell Biol 2011; 136(4): 437-54.

[68] Tiedemann K, Batge B, Muller PK, Reinhardt DP. Interactions of fibrillin-1 with heparin/heparan sulfate, implications for microfibrillar assembly. J Biol Chem 2001; 276(38): 36035-42.

[69] Parsi MK, Adams JR, Whitelock J, Gibson MA. LTBP-2 has multiple heparin/heparan sulfate binding sites. Matrix Biol 2010; 29(5): 393-401.

[70] Massam-Wu T, Chiu M, Choudhury R, *et al.* Assembly of fibrillin microfibrils governs extracellular deposition of latent TGF beta. J Cell Sci 2010; 123(Pt 17): 3006-18.

[71] Sabatier L, Djokic J, Hubmacher D, Dzafik D, Nelea V, Reinhardt DP. Heparin/heparan sulfate controls fibrillin-1, -2 and -3 self-interactions in microfibril assembly. FEBS Lett 2014; 588(17): 2890-7.

[72] Tiedemann K, Sasaki T, Gustafsson E, *et al.* Microfibrils at basement membrane zones interact with perlecan *via* fibrillin-1. J Biol Chem 2005; 280(12): 11404-12.

[73] Ritty TM, Broekelmann TJ, Werneck CC, Mecham RP. Fibrillin-1 and -2 contain heparin-binding sites important for matrix deposition and that support cell attachment. Biochem J 2003; 375(Pt 2): 425-32.

[74] Pan W, Miao HQ, Xu YJ, *et al.* 1-[4-(1H-Benzoimidazol-2-yl)-phenyl]-3-[4-(1H-benzoimidazol-2-yl)-phenyl]-urea derivatives as small molecule heparanase inhibitors. Bioorg Med Chem Lett 2006; 16(2): 409-12.

[75] Iriyama S, Tsunenaga M, Amano S, Adachi E. Key role of heparan sulfate chains in assembly of anchoring complex at the dermal-epidermal junction. Exp Dermatol 2011; 20(11): 953-5.

[76] Rodrigo FG, Cotta-Pereira G. Connective fibers involved in the dermoepidermal anchorage. An electron microscopical study. Dermatologica 1979; 158(1): 13-23.

[77] Iriyama S, Umishio K, Tsunenaga M, Inomata S, Adachi E, inventor; Shiseido Company Ltd., assignee. Artificial skin. Patent WO2011108300 A1. 2010 Mar.

[78] Hook M, Wasteson A, Oldberg A. A heparan sulfate-degrading endoglycosidase from rat liver tissue. Biochem Biophys Res Commun 1975; 67(4): 1422-8.

[79] Nakajima M, Irimura T, Di Ferrante N, Nicolson GL. Metastatic melanoma cell heparanase. Characterization of heparan sulfate degradation fragments produced by B16 melanoma endoglucuronidase. J Biol 1984; 259(4): 2283-90.

[80] Ricoveri W, Cappelletti R. Heparan sulfate endoglycosidase and metastatic potential in murine fibrosarcoma and melanoma. Cancer Res 1986; 46(8): 3855-61.

[81] Lerner I, Zcharia E, Neuman T, *et al.* Heparanase is preferentially expressed in human psoriatic lesions and induces development of psoriasiform skin inflammation in mice. Cell Mol Life Sci 2014; 71(12): 2347-57.

[82] Naparstek Y, Cohen IR, Fuks Z, Vlodavsky I. Activated T lymphocytes produce a matrix-degrading heparan sulphate endoglycosidase. Nature 1984; 310(5974): 241-4.

[83] Freeman C, Browne AM, Parish CR. Evidence that platelet and tumour heparanases are similar enzymes. Biochem J 1999; 342 (Pt 2): 361-8.

[84] Bernard D, Mehul B, Delattre C, Simonetti L, Thomas-Collignon A, Schmidt R. Purification and characterization of the endoglycosidase heparanase 1 from human plantar stratum corneum: a key enzyme in epidermal physiology? J Invest Dermatol 2001; 117(5): 1266-73.

[85] Iriyama S, Matsunaga Y, Amano S. Heparanase activation induces epidermal hyperplasia, angiogenesis, lymphangiogenesis and wrinkles. Exp Dermatol 2010; 19(11): 965-72.

[86] Ohkawa T, Naomoto Y, Takaoka M, *et al.* Localization of heparanase in esophageal cancer cells: respective roles in prognosis and differentiation. Lab Invest 2004; 84(10): 1289-304.

[87] Gingis-Velitski S, Ishai-Michaeli R, Vlodavsky I, Ilan N. Anti-heparanase monoclonal antibody enhances heparanase enzymatic activity and facilitates wound healing. FASEB J 2007; 21(14): 3986-93.

[88] Malgouries S, Donovan M, Thibaut S, Bernard BA. Heparanase 1: a key participant of inner root sheath differentiation program and hair follicle homeostasis. Exp Dermatol 2008; 17(12): 1017-23.

[89] Coulson-Thomas VJ, Gesteira TF, Esko J, Kao W. Heparan sulfate regulates hair follicle and sebaceous gland morphogenesis and homeostasis. J Biol Chem 2014; 289(36): 25211-26.

[90] Fairbanks MB, Mildner AM, Leone JW, *et al.* Processing of the human heparanase precursor and evidence that the active enzyme is a heterodimer. J Biol Chem 1999; 274(42): 29587-90.

[91] Shafat I, Vlodavsky I, Ilan N. Characterization of mechanisms involved in secretion of active heparanase. J Biol Chem 2006; 281(33): 23804-11.

[92] Toyoshima M, Nakajima M. Human heparanase. Purification, characterization, cloning, and expression. J Biol Chem 1999; 274(34): 24153-60.

[93] Vlodavsky I, Friedmann Y, Elkin M, *et al.* Mammalian heparanase: gene cloning, expression and function in tumor progression and metastasis. Nat Med 1999; 5(7): 793-802.

[94] Nakajima M, Irimura T, Nicolson GL. Heparanases and tumor metastasis. J Cell Biochem 1988; 36(2): 157-67.
[95] Reinheckel T, Hagemann S, Dollwet-Mack S, *et al.* The lysosomal cysteine protease cathepsin L regulates keratinocyte proliferation by control of growth factor recycling. J Cell Sci 2005; 118(Pt 15): 3387-95.
[96] Riteau N, Baron L, Villeret B, *et al.* ATP release and purinergic signaling: a common pathway for particle-mediated inflammasome activation. Cell Death Dis 2012; 3: e403.
[97] Communi D, Janssens R, Suarez-Huerta N, Robaye B, Boeynaems JM. Advances in signalling by extracellular nucleotides. the role and transduction mechanisms of P2Y receptors. Cell Signal 2000; 12(6): 351-60.
[98] Inoue K, Hosoi J, Denda M. Extracellular ATP has stimulatory effects on the expression and release of IL-6 *via* purinergic receptors in normal human epidermal keratinocytes. J Invest Dermatol 2007; 127(2): 362-71.
[99] Tammi RH, Hyyrylainen AM, Maibach HI, Tammi MI. Ultrastructural localization of keratinocyte surface associated heparan sulphate proteoglycans in human epidermis. Histochemistry 1987; 87(3): 243-50.
[100] Dreyfuss JL, Regatieri CV, Jarrouge TR, Cavalheiro RP, Sampaio LO, Nader HB. Heparan sulfate proteoglycans: structure, protein interactions and cell signaling. An Acad Bras Cienc 2009; 81(3): 409-29.
[101] Dietrich CP, Nader HB, Straus AH. Structural differences of heparan sulfates according to the tissue and species of origin. Biochem Biophys Res Commun 1983; 111(3): 865-71.
[102] Freeman C, Liu L, Banwell MG, *et al.* Use of sulfated linked cyclitols as heparan sulfate mimetics to probe the heparin/heparan sulfate binding specificity of proteins. J Biol Chem 2005; 280(10): 8842-9.
[103] Kjellen L, Lindahl U. Proteoglycans: structures and interactions. Annu Rev Biochem 1991; 60: 443-75.
[104] Rops AL, van der Vlag J, Lensen JF, *et al.* Heparan sulfate proteoglycans in glomerular inflammation. Kidney Int 2004; 65(3): 768-85.
[105] Vlodavsky I, Friedmann Y. Molecular properties and involvement of heparanase in cancer metastasis and angiogenesis. J Clin Invest 2001; 108(3): 341-7.
[106] Rapraeger AC, Krufka A, Olwin BB. Requirement of heparan sulfate for bFGF-mediated fibroblast growth and myoblast differentiation. Science 1991; 252(5013): 1705-8.
[107] Yayon A, Klagsbrun M, Esko JD, Leder P, Ornitz DM. Cell surface, heparin-like molecules are required for binding of basic fibroblast growth factor to its high affinity receptor. Cell 1991; 64(4): 841-8.
[108] Elkin M, Ilan N, Ishai-Michaeli R, *et al.* Heparanase as mediator of angiogenesis: mode of action. FASEB J 2001; 15(9): 1661-3.
[109] Okawa T, Naomoto Y, Nobuhisa T, *et al.* Heparanase is involved in angiogenesis in esophageal cancer through induction of cyclooxygenase-2. Clin Cancer Res 2005; 11(22): 7995-8005.
[110] Patel VN, Knox SM, Likar KM, *et al.* Heparanase cleavage of perlecan heparan sulfate modulates FGF10 activity during ex vivo submandibular gland branching morphogenesis. Development 2007; 134(23): 4177-86.
[111] Detmar M, Yeo KT, Nagy JA, *et al.* Keratinocyte-derived vascular permeability factor (vascular endothelial growth factor) is a potent mitogen for dermal microvascular endothelial cells. J Invest Dermatol 1995; 105(1): 44-50.
[112] Skobe M, Hawighorst T, Jackson DG, *et al.* Induction of tumor lymphangiogenesis by VEGF-C promotes breast cancer metastasis. Nat Med 2001; 7(2): 192-8.
[113] Ashikari-Hada S, Habuchi H, Kariya Y, Itoh N, Reddi AH, Kimata K. Characterization of growth factor-binding structures in heparin/heparan sulfate using an octasaccharide library. J Biol Chem 2004; 279(13): 12346-54.
[114] Perrimon N, Bernfield M. Specificities of heparan sulphate proteoglycans in developmental processes. Nature 2000; 404(6779): 725-8.
[115] Kan M, Wu X, Wang F, McKeehan WL. Specificity for fibroblast growth factors determined by heparan sulfate in a binary complex with the receptor kinase. J Biol Chem 1999; 274(22): 15947-52.
[116] Kreuger J, Salmivirta M, Sturiale L, Gimenez-Gallego G, Lindahl U. Sequence analysis of heparan sulfate epitopes with graded affinities for fibroblast growth factors 1 and 2. J Biol Chem 2001; 276(33): 30744-52.
[117] Luo Y, Ye S, Kan M, McKeehan WL. Control of fibroblast growth factor (FGF) 7- and FGF1-induced mitogenesis and downstream signaling by distinct heparin octasaccharide motifs. J Biol Chem 2006; 281(30): 21052-61.
[118] Tammela T, He Y, Lyytikka J, *et al.* Distinct architecture of lymphatic vessels induced by chimeric vascular endothelial growth factor-C/vascular endothelial growth factor heparin-binding domain fusion proteins. Circ Res 2007; 100(10): 1468-75.
[119] Kiryushko D, Novitskaya V, Soroka V, *et al.* Molecular mechanisms of Ca(2+) signaling in neurons induced by the S100A4 protein. Mol Cell Biol 2006; 26(9): 3625-38.
[120] Donato R, Cannon BR, Sorci G, *et al.* Functions of S100 proteins. Curr Mol Med 2013; 13(1): 24-57.
[121] Robinson MJ, Tessier P, Poulsom R, Hogg N. The S100 family heterodimer, MRP-8/14, binds with high affinity to heparin and heparan sulfate glycosaminoglycans on endothelial cells. J Biol Chem 2002; 277(5): 3658-65.
[122] Hirobe T, Osawa M, Nishikawa S. Steel factor controls the proliferation and differentiation of neonatal mouse epidermal melanocytes in culture. Pigment Cell Res 2003; 16(6): 644-55.
[123] Hirobe T, Osawa M, Nishikawa S. Hepatocyte growth factor controls the proliferation of cultured epidermal melanoblasts and melanocytes from newborn mice. Pigment Cell Res 2004; 17(1): 51-61.
[124] Hirobe T, Furuya R, Hara E, Horii I, Tsunenaga M, Ifuku O. Granulocyte-macrophage colony-stimulating factor (GM-CSF) controls the proliferation and differentiation of mouse epidermal melanocytes from pigmented spots induced by ultraviolet radiation B. Pigment Cell Res 2004;17(3): 230-40.
[125] Christiano AM, Rosenbaum LM, Chung-Honet LC, *et al.* The large non-collagenous domain (NC-1) of type VII collagen is amino-terminal and chimeric. Homology to cartilage matrix protein, the type III domains of fibronectin and the A domains of von Willebrand factor. Human molecular genetics. 1992;1(7):475-81.
[126] Iriyama S, Hiruma T, Tsunenaga M, Amano S. Influence of heparan sulfate chains in proteoglycan at the dermal-epidermal junction on epidermal homeostasis. Exp Dermatol 2011; 20(10): 810-4.
[127] Pineau N, Bernerd F, Cavezza A, Dalko-Csiba M, Breton L. A new C-xylopyranoside derivative induces skin expression of glycosaminoglycans and heparan sulphate proteoglycans. Eur J Dermatol 2008; 18(1): 36-40.
[128] Sahuc F, Nakazawa K, Berthod F, Collombel C, Damour O. Mesenchymal-epithelial interactions regulate gene expression of type VII collagen and kalinin in keratinocytes and dermal-epidermal junction formation in a skin equivalent model. Wound Repair Regen 1996; 4(1): 93-102.
[129] Breitkreutz D, Stark HJ, Mirancea N, Tomakidi P, Steinbauer H, Fusenig NE. Integrin and basement membrane normalization in mouse grafts of human keratinocytes--implications for epidermal homeostasis. Differentiation 1997; 61(3): 195-209.
[130] Canty EG, Lu Y, Meadows RS, Shaw MK, Holmes DF, Kadler KE. Coalignment of plasma membrane channels and protrusions (fibripositors) specifies the parallelism of tendon. J Cell Biol 2004; 165(4): 553-63.
[131] Canty EG, Starborg T, Lu Y, *et al.* Actin filaments are required for fibripositor-mediated collagen fibril alignment in tendon. J Biol Chem 2006; 281(50): 38592-8.
[132] Humphries SM, Lu Y, Canty EG, Kadler KE. Active negative control of collagen fibrillogenesis *in vivo*. Intracellular cleavage of the type I procollagen propeptides in tendon fibroblasts without intracellular fibrils. J Biol Chem 2008; 283(18): 12129-35.
[133] Pellegrini G, Ranno R, Stracuzzi G, *et al.* The control of epidermal stem cells (holoclones) in the treatment of massive full-thickness burns with autologous keratinocytes cultured on fibrin. Transplantation 1999; 68(6): 868-79.
[134] Ronfard V, Rives JM, Neveux Y, Carsin H, Barrandon Y. Long-term regeneration of human epidermis on third degree burns transplanted with autologous cultured epithelium grown on a fibrin matrix. Transplantation 2000; 70(11): 1588-98.
[135] Kapacee Z, Richardson SH, Lu Y, *et al.* Tension is required for fibripositor formation. Matrix Biol 2008; 27(4): 371-5.
[136] Boehnke K, Mirancea N, Pavesio A, Fusenig NE, Boukamp P, Stark HJ. Effects of fibroblasts and microenvironment on epider-

mal regeneration and tissue function in long-term skin equivalents. Eur J Cell Biol 2007; 86(11-12): 731-46.

[137] Yannas IV, Burke JF, Gordon PL, Huang C, Rubenstein RH. Design of an artificial skin. II. Control of chemical composition. J Biomed Mater Res 1980; 14(2): 107-32.

[138] Yannas IV, Lee E, Orgill DP, Skrabut EM, Murphy GF. Synthesis and characterization of a model extracellular matrix that induces partial regeneration of adult mammalian skin. Proc Natl Acad Sci USA 1989; 86(3): 933-7.

[139] Michel M, L'Heureux N, Pouliot R, Xu W, Auger FA, Germain L. Characterization of a new tissue-engineered human skin equivalent with hair. In Vitro Cell Dev Biol Anim 1999; 35(6): 318-26.

[140] Auger FA, Berthod F, Moulin V, Pouliot R, Germain L. Tissue-engineered skin substitutes: from *in vitro* constructs to *in vivo* applications. Biotechnol Appl Biochem 2004; 39(Pt 3): 263-75.

[141] Pouliot R, Larouche D, Auger FA, *et al.* Reconstructed human skin produced *in vitro* and grafted on athymic mice. Transplantation 2002; 73(11): 1751-7.

[142] Asano Y, Nishiguchi A, Matsusaki M, *et al.* Ultrastructure of blood and lymphatic vascular networks in three-dimensional cultured tissues fabricated by extracellular matrix nanofilm-based cell accumulation technique. Microscopy (Oxf) 2014; 63(3): 219-26.

[143] Parnigotto PP, Bernuzzo S, Bruno P, Conconi MT, Montesi F. Characterization and applications of human epidermis reconstructed *in vitro* on de-epidermized derma. Farmaco 1998; 53(2): 125-31.