

Effect of Glucosamine-Derivate Molecules on the Extracellular Matrix Remodeling In Human Dermal Fibroblast

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Abstract

Skin is the largest human organ that undergoes alterations due to the passage of time. It is subject to direct environmental challenge; especially the most common source of damage is solar ultraviolet (UV) irradiation. The photoaging affects the phenotype of different cellular type such as keratinocytes, fibroblasts and dendritic cells either by direct effects of irradiation on the cells or indirectly by the remodel and aged extracellular matrix (ECM). Inappropriate ECM composition is involved in several skin pathologies as well as in skin aging. Previous studies in our lab have been focused on the effect of glucosamine (GlcN) and an its peptidyl-derivatives 2-(N-Acetyl)-L-phenylalanyl-amido-2-deoxy- β -D-glucose (NAPA) and 2-(N-Carbobenzyloxy)-L-phenylalanyl-amido-2-deoxy- β -D-glucose (NCPA), to induce ECM production and the inhibition of IKK α activation in human dermal fibroblasts, as potential new drug for aging and skin disorders treatment.

Editorial

Skin represents the major organ in which aging-related changes are visible [1]. The appearance of skin reflects general health and communicates ethnicity, lifestyle and age. Like all organs of the body, undergoes alterations, not only by passing time alone (intrinsic aging) but also by the exposure to different environmental factors (extrinsic aging). The most important environmental factor leading to extrinsically aged skin is solar radiation [2]. The detrimental effect of solar radiation on the facial appearance regarding skin aging was already recognized by the dermatologists Unna and Dubreuilh [3,4] in the late 19th century, comparing the skin of sailors and farmers to that of indoor workers. The process of aging affects the skin in multiple ways and involves both its layers: epidermis and dermis. Dermis, which provides structural support for the skin's vasculature, appendages and epidermis, is composed primarily of extracellular matrix (ECM) which is an intricate network of macromolecules. This ECM is made up of several types of proteins, proteoglycans, and glycosaminoglycans, which are largely produced and secreted by fibroblasts. Type I collagen (Coll I) is by far the most abundant protein in human skin, comprising greater than 90% of its dry weight. The unique physical properties of collagen fibers together with ECM confer structural integrity to skin [5].

Fibroblasts are the most abundant cell type within all the body's connective tissue, and their primary role is the secretion of components of ECM. Inappropriate ECM composition is involved in several skin pathologies as well as in skin aging. For instance, human aged skin shows histological and biochemical changes, such as thinning of epidermis, atrophy and flattening of the dermo-epidermal

junction. In dermis, collagen fibers appear thickened and fragmented with higher ratio of Coll III to Coll I, and polysaccharide and proteoglycans of ECM are reported with abnormal localization and structure. All these changes cause an inefficient dermal hydration leading to clinical manifestation such as dry, fragile skin and wrinkle formation [6]. Dermal fibroblasts are involved also in formation of keloids and hypertrophic scarring. These processes are characterized by excessive ECM production, especially Coll I, persistence of myofibroblasts, increased expression of α -smooth muscle actin (α -SMA) and various cytokines such as TGF- β . The main cause of collagen degradation is the cleavage by metalloproteinase (MMPs) and accordingly, the non-complete *de novo* reconstitution of collagen synthesis. The repeated damage of collagen network is caused by chronic exposure to solar radiation. Furthermore this photoaging also affects the phenotype of other embedded cells such as keratinocytes, fibroblasts and dendritic cells either by direct effects of irradiation on the cells or indirectly by the remodel and aged ECM. Little is known about the signaling events following UVB (280-320 nm wavelengths) irradiation [7]. Dong et al. [8] demonstrated that UVB stimulation induces c-fos upregulation by nuclear IKK α -mediated histone H3 phosphorylation in a NF- κ B-independent way. I κ B kinase (IKK) complex, the master kinase for NF- κ B activation, contains two subunits, IKK α and IKK β , that share structural and biochemical similarities, but differs in sub-cellular localization and phosphorylation targets and then, they have a distinct physiological and pathological roles. While IKK β is predominantly cytoplasmatic, IKK α has been found to shuttle between the cytoplasm and the nucleus where it plays a role in chromatin regulation not only to NF- κ B target genes but also affects gene transcription regulated by various transcription factors or cofactors [9]. Previous studies in our lab have been focused on the effect of glucosamine (GlcN) and an its peptidyl-derivative 2-(N-Acetyl)-L-phenylalanyl-amido-2-deoxy- β -D-glucose (NAPA), synthesized in our laboratory (patent: RM 2009 A000369) [10], to induce ECM production in cartilage as potential new drug for osteoarthritis treatment. The *in vivo* and *in vitro* studies, conducted in a rabbit experimental osteoarthritis model and in three-dimensional model of chondrocyte culture, micro-masses, after administering GlcN and NAPA showed that both molecules were able to stimulate ECM component such as Collagen type II, hyaluronic acid and some non-collagenous components, as Biglycan, Lumican and Decorin [11]. Starting from glucosamine, we designed a new and more selective molecule, 2-(N-Carbobenzyloxy)-L-phenylalanyl-amido-2-deoxy- β -D-glucose (NCPA) that worked at lower concentration than glucosamine because more hydrophobic, and were able to inhibit IKK α nuclear translocation and its activity, but did not affect IKK β activity. Furthermore, NAPA and GlcN were able to interfere with AP-1 pathway by inhibiting p38 and JNK kinases and consequently by

inhibiting MMP-1, -3 and -13 production [12-14]. The successful results have prompting us to analyze if this molecules could be able to induce ECM production and to inhibit IKK α activation followed by UVB irradiation also in other connective tissue, as skin, to exert an anti-aging effect and prevention from photodamage.

Firstly we started the experiment isolating human fibroblasts from biopsy skin specimens from donors with both informed donor consent and Human Research Ethics Committee approval. Fibroblasts were exposed to a dose of 0.5 kJ/m² UVB for 30 minutes to have a greater increasing of phospho-IKK α into the nucleus. A pretreatment with NAPA and NCPA has proven effective to inhibit this activation. As a nuclear protein, IKK α has been demonstrated to act as chromatin-associated kinase that can phosphorylate different targets in the promoter region of many genes. So, its kinase activity is critical for its function. Since nuclear IKK α may function as a common epigenetic regulator for gene transcription, we investigated the expression level of molecules under the transcriptional control of IKK α proteins founding the ability of NAPA and NCPA to inhibit the phosphorylation of Histone H3 at Ser10 and CBP at Ser1382/1386 and p65 at S536. The impairments of extracellular matrix (ECM) that lead to a skin disorders, was restored in dermal fibroblasts after treatment with NAPA and NCPA that showed a significant increase in the ratio of type I collagen to type III.

Now-a-days there exist many molecules that show moderate or high promiscuity for the two homologous kinases. Due to their differentiated roles in NF- κ B signaling cascade, selective inhibition of IKK α and IKK β can result in different biological effects on physiological and pathological processes implicated in the NF- κ B pathway. To date, a variety of smallmolecule inhibitors have been developed for specifically targeting IKK complex. However, although many potent IKK inhibitors have been discovered over the past decades, only very few of them exhibit high selectivity between IKK α and IKK β . Up to date, rational design of selective IKK inhibitors is still a great challenge since the two homologous kinases share high structural conservation around their active sites and also because the molecular mechanism of ligand interactions with IKK α and IKK β still remains largely unexplored.

References

1. Zouboulis CC, Boschnakow A (2001) Chronical ageing and photoageing of the human sebaceous gland. *Clin Exp Dermatol* 26: 600-607.
2. Tigges J, Krutmann J, Fritsche E, Haendeler J, Schaal H, et al. (2014) The hallmarks of fibroblasts ageing. *Mechanisms of Ageing and Development* 138: 26-44.
3. Unna PG (1894) Die Histopathologie der Hautkrankheiten. In: J. Orth: Lehrbuch der speziellen pathologischen Anatomie. Berlin: August Hirschwald.
4. Dubreuilh W (1896) Des hyperkeratoses circumscriptes. *Ann Derm et Syph* 7:1158-1204.
5. Fisher GJ, Varani J, Voorhees J (2008) Looking older: Fibroblast collapse and therapeutic implications. *Arch Dermatol* 144: 666-672.
6. Brun C, Jean-Louis F, Oddos T, Bagot M, Bensussan A, et al. (2016) Phenotypic and functional changes in dermal primary fibroblasts isolated from intrinsically aged human skin. *Exp Dermatol* 25: 113-119.
7. Rock K, Grandoch M, Majora M, Krutmann J, Fischer JW (2011) Collagen fragments inhibit hyaluronan synthesis in skin fibroblasts in response to Ultraviolet B (UVB). New insights into mechanisms of matrix remodeling. *Journal of Biological Chemistry* 286: 18268-18276.
8. Dong W, Li Y, Gao M, Hu M, Li X, et al. (2011) IKK α contributes to UVB induces VEGF expression by regulating AP-1 transactivation. *Nucleic Acid Research* 40: 2940-2955.
9. Huang WC, Hung MC (2013) Beyond NF- κ B activation: nuclear functions of I κ B kinase α . *Journal of Biomedical Sciences* 20: 3.
10. Giordano C, Gallina C, Consalvi V, Scandurra R (1991) Synthesis and properties of D-glucosamine Npeptidyl derivatives as substrate analog inhibitors of papain and cathepsin B. *Eur J Med Chem* 26: 753-762.
11. Scotto d'Abusco A, Corsi A, Grillo MG, Cicione C, Calamia V, et al. (2008) Effects of intra-articular administration of glucosamine and a peptidyl-glucosamine derivative in a rabbit model of experimental osteoarthritis: a pilot study. *Rheumatol Int* 28: 437-443.
12. Scotto d'Abusco A, Calamia V, Cicione C, Grigolo B, Politi L, et al. (2007) Glucosamine affects intracellular signalling through inhibition of mitogen-activated protein kinase phosphorylation in human chondrocytes. *Arthritis Res Ther* 9: R104.
13. Scotto d'Abusco A, Politi L, Giordano C, Scandurra R (2010) A peptidyl-glucosamine derivative affects IKK α kinase activity in human chondrocytes. *Arthritis Res Ther* 12: R18.
14. Stoppoloni D, Politi L, Leopizzi M, Gaetani S, Guazzo R, et al. (2015) Effect of glucosamine and its peptidyl-derivative on the production of extracellular matrix components by human primary chondrocytes. *Osteoarthritis Cartilage* 23: 103-113.