Expression of Hyaluronan Synthase and Collagen Type I mRNA by Hyaluronan Tetrasaccharides in Normal Human Dermal Fibroblasts

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(Accepted: December 3, 2012)

Abstract

The major compounds in the dermis are hyaluronan and type I collagen, which decrease with aging, UV and various other factors. The loss of hyaluronan and collagen with aging is associated with increased dehydration and wrinkling of the skin. We aimed to investigate the influence of hyaluronan tetrasaccharide (HA4) on regulation of high molecular weight hyaluronan (HA) and collagen synthesis in normal human dermal fibroblasts (NHDFs). Expression of hyaluronan synthase (HAS) 1–3 and collagen (COL) I A1 mRNA were evaluated by quantitative real-time PCR. HAS1 mRNA expression was found to be increased in NHDFs treated with HA and HA4. In addition, it was observed that NHDFs co-cultured with normal human epidermal keratinocytes (NHEKs) showed up-regulation of HAS1 mRNA expression, as compared with NHDFs single cell culture treated with HA4. Treatment of NHDFs with HA4 + derivatized vitamin C (VC-PMg) significantly increased COL1A1 mRNA expression. In this study, we confirmed that HA4 affected HAS1 and COL1A1 mRNA expression; thus, HA4 application may show various action in skin.

Key words: hyaluronan tetrasaccharide, hyaluronan synthase, collagen, mRNA.

1. Introduction

Hyaluronan, which is composed of repeated β-1,4-glucuronic acid-β-1,3-N-acetylglucosamine disaccharide units, is a non-sulfated glycosaminoglycan with a molecular weight of over 1,000 kDa. Hyaluronan is abundant in the extracellular matrix (ECM) of the skin, and is involved in many biological processes such as tissue homeostasis, cell proliferation, cell migration, cell differentiation, angiogenesis, tumor biology and repair processes by interacting with various proteins. In addition, because of physical properties such as viscoelasticity, hydrophilicity and extensibility, hyaluronan seems to play an important role as a space filler, osmotic buffer, plasma protein sieve and lubricant. In the skin, hyaluronan synthases (HAS) are synthesized by dermal fibroblasts and epidermal keratinocytes. Synthesis of hyaluronan is accomplished by three HAS (HAS1, HAS2 and HAS3), which produce different sizes of polysaccharide chains with average molecular weights of about 3 × 10⁵ to 2 × 10⁶ Da for HAS1 and HAS2, and about 2 × 10⁶ to 3 × 10⁷ Da for HAS3.

Collagen is a major component of ECM, and type I collagen accounts for approximately 80% of the total collagen in adult human dermis. Type I collagen is a product of two genes, α1 (I) (COL1A1) and α2 (I) (COL1A2), which are coordinately regulated. The major structural protein in the dermis is type I collagen, which comprises 90% of the dry weight of skin and diminishes with normal aging. Collagen plays a role in cell adhesion, and is important for maintaining normal tissue architecture and function.

Stimulation of living tissue by hyaluronan appears to depend on various factors, including hyaluronan chain length. For example, high molecular weight hyaluronan was demonstrated to suppress angiogenesis and inflammation, whereas low molecular weight hyaluronan stimulated induction of angiogenesis, inflammation and expression heat shock protein 72. In addition, injection of cross-linked hyaluronan stimulates collagen synthesis, partially restoring dermal matrix components that are lost in photo-damaged skin. In this way, hyaluronan possesses numerous functions, but little is known about the effects of hyaluronan oligosaccharides on skin.

In this study, we aimed to investigating the influence of high molecular weight hyaluronan (HA) and hyaluronan tetrasaccharide (HA4) on regulation of HASs and COL1A1 mRNA expression in normal human dermal fibroblasts (NHDFs).

2. Materials and Methods

2–1. Materials

HA4 (99.14%) (776.3 Da) was provided by Glycoscience Laboratories Inc. (Tokyo, Japan). HA (>1,200 kDa) produced by a Streptococcus zooepidemicus was used. All other chemicals and solvents were of analytical grade. Abdominal skin origin normal human dermal fibroblasts (NHDFs) were purchased from Kurabo (Osaka, Japan). Abdominal skin origin normal human epidermal keratinocytes (NHEKs) were...